Active Concepts

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Bio-Active Algal Lipids: The Next Generation Cellular Modulators

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Overview

Survival Mechanism

Sustainability

Algal Metabolites

Mechanism of Retinol & Ceramides

Skin Genomics Assay

Survival

Algae are photosynthetic organisms that grow in a range of aquatic habitats such as lakes, ponds, rivers, oceans and even wastewater.

Incredible survival mechanisms allow different species of algae to thrive in fresh-water and salt-water globally. Hostile environments, extreme temperatures, UV radiation, and pollution, empower algae with unique phytochemicals, proteins, antioxidants, and bioactive lipids to resist environmental damage while having the ability to recover and regenerate completely.

Environmental variance is critical for algae specialization and survival, including light intensity, temperature, salinity, pH fluctuation, CO2 levels, and nutrient content.

Algae utilizes atmospheric CO2 to convert potential nutrients such as nitrogen, phosphorus and carbon into bioactive secondary metabolites. These secondary metabolites provide themselves with a form of chemical defense against other invasive micro-organisms and predators.

They play a role in preventing bacterial biofilm formation and even the adherence of other algae species. This competitive inhibition mechanism of biofilms is not due to toxic nor growth-retarding ability of algae, instead, algal secondary metabolites block bacterial receptors to prevent communication or quorum sensing, which is enabled via biofilms.

Defense mechanism employed by algae can effectively inhibit marine fouling. (2017, February 2). Retrieved from https://www.sciencedaily.com/releases/2017/02/170202141033.htm
Khan, M. I., Shin, J. H., & Kim, J. D. (2018, March 5). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products.

Sustainability

A large number of benefits of algae stem from the fact that these species of plant have evolved over millions of years to produce and store energy in the form of oil.

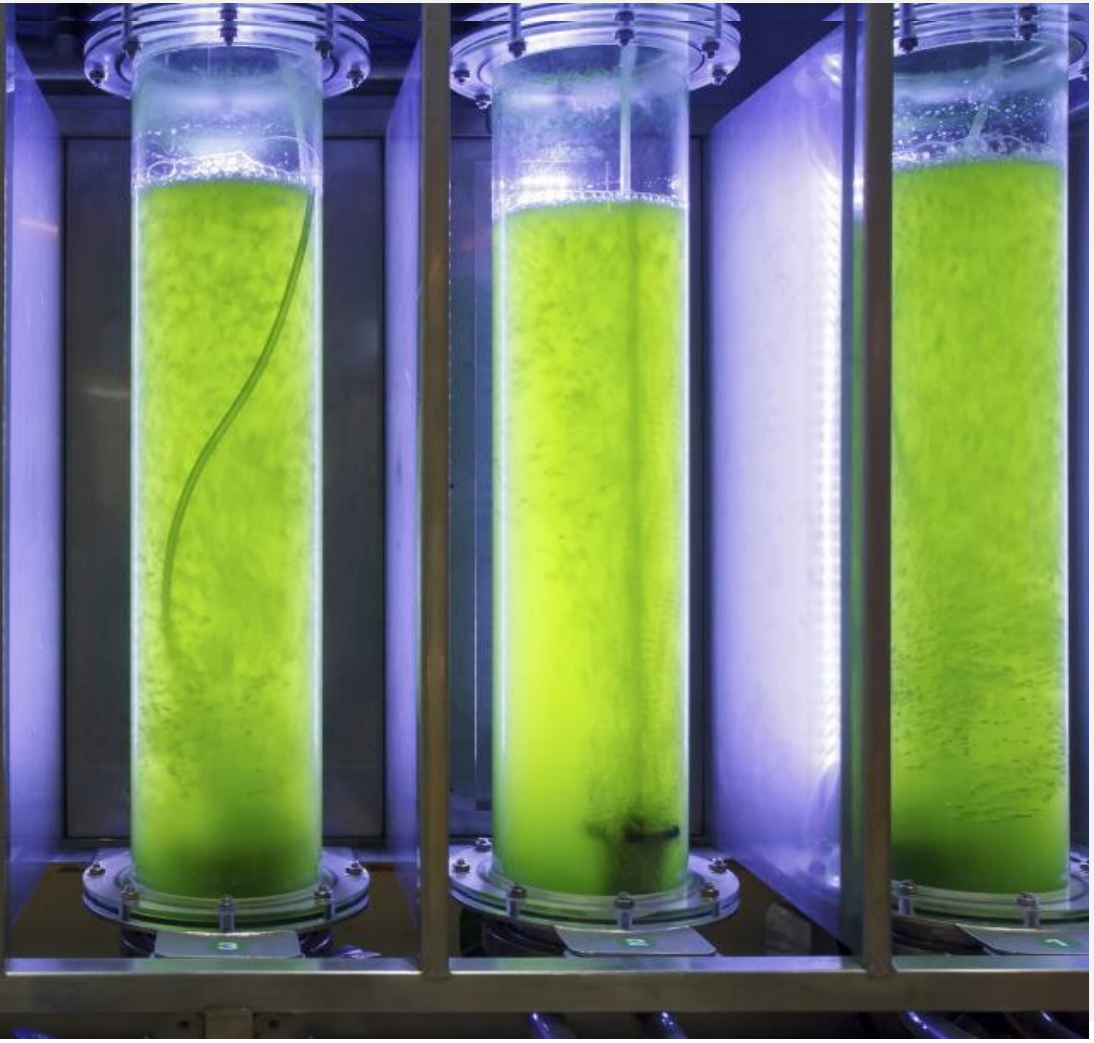
Microalgae are able to grow faster than many land crops and can double their number within a few hours producing a high yield of biomass. The cultivation of microalgae utilizes the land that is unsuitable for traditional agriculture eliminating the need for deforestation.

Microalgae thrive in nutrient-rich waters like municipal waste waters, animal wastes and some industrial effluents, at the same time purifying these wastes while producing a biomass suitable for biofuels production.

The global algae biofuel market is expected to reach USD 10.73 billion by 2025, according to a new report by Grand View Research, Inc. Depleting fossil fuel resources as well as rising awareness towards environment protection is expected to be the key factor for driving industry growth.

Khan, M. I., Shin, J. H., & Kim, J. D. (2018, March 5). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products.

Algae in Global Industries



Microalgae have recently attracted considerable interest worldwide, due to their extensive application potential in the renewable energy, biopharmaceutical, and nutraceutical industries.

Algae is renewable, sustainable and economical sources of:

- Biofuels
- Bioactive medicinal products
- Food ingredients such as proteins, vitamins, antioxidants, lipids, and polysaccharides
- Liquid fossil fuels

Khan, M. I., Shin, J. H., & Kim, J. D. (2018, March 5). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products.

Chlamydomonas reinhardtii

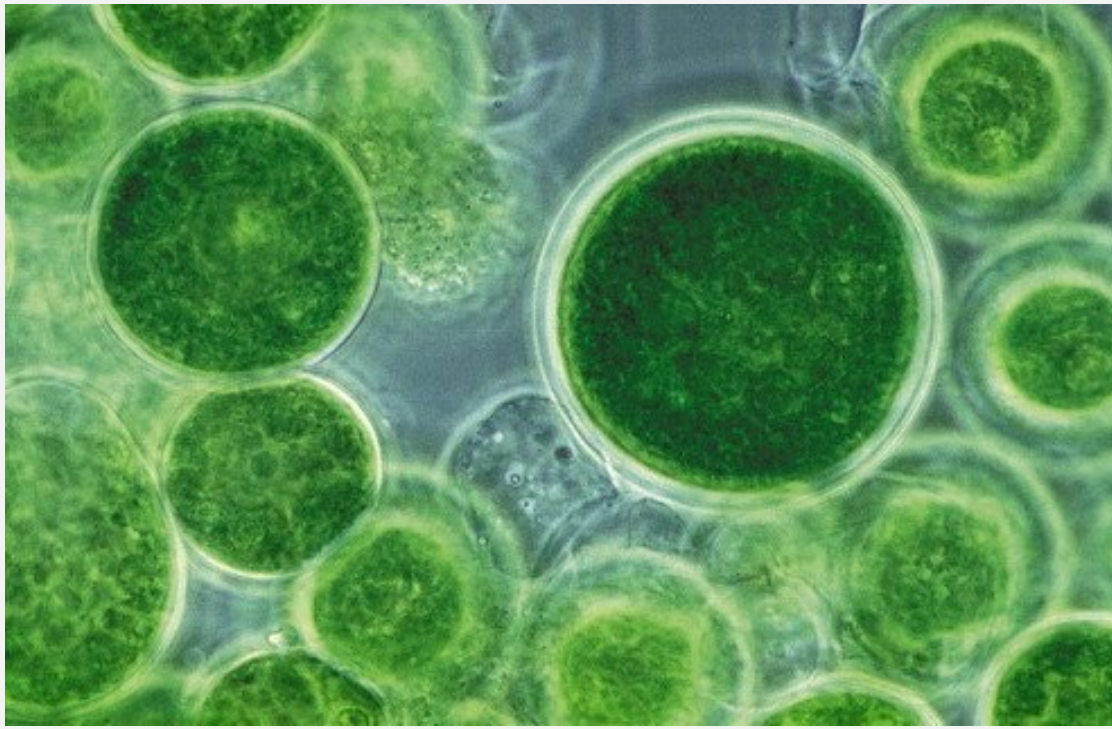
C. reinhardtii - Lipids

C. reinharditii is a single celled microalgae with membrane bound organelles that swims with two flagella.

This species of green algae, is particularly capable of producing high amounts of lipids including essential fatty acids, sterols, and even phytoceramides.

The average composition of lipid bodies in *C. reinhardtii*:

- 90% TAG (triacylglicerides) –composed of 50% palmitic and oleic acids and 50% unsaturated fatty acids half of which are oleic acid.
- 10% free fatty acids- composed of palmitic and oleic acids



Popko, J. (1970, January 1). Lipid Composition of Chlamydomonas reinhardtii.
Kong, J. N., Hardin, K., Dinkins, M., Wang, G., He, Q., Mujadzic, T., … Bieberich, E. (2015, December 1). Regulation of Chlamydomonas flagella and ependymal cell motile cilia by ceramide-mediated translocation of GSK3.
Salehi-Ashtiani, K., & Papin, J. A. (2012, January 13). Experimental Definition and Validation of Protein Coding Transcripts in Chlamydomonas reinhardtii.
ZiTeng Wang, Nico Ullrich, Sunjoo Joo, Sabine Waffenschmidt, Ursula Goodenouh Eukaryotic Cell Dec 2009, 8 (12) 1856-1868

Structural and Functional Features

This photosynthetic species of green algae employs diverse strategies of regulation and photoprotection to avoid, minimize, and repair photo-oxidative damage in stressful light conditions, allowing for acclimation to different and changing environments.

C. reindharditii has great potential to be used as an alternative energy source. Its biochemical aspects are currently being studied with the target to increase its energy production rate and/or sustainability. However, there is still quite fragmentary knowledge concerning the cell's precise structural properties.

In effort to explore the structural composition and intracellular processes of this species, a study published in Journal of Integrative Bioinformatics, have looked at Heuristic Modeling and 3D Stereoscopic Visualization of a *Chlamydomonas reinhardtii* Cell. As a result, cell wall, membranes, and organelles were identified along with lipid bodies which occupy a large portion of the cells.

Biere, N., Ghaffar, M., Doebbe, A., Jäger, D., Rothe, N., Friedrich, B. M., … Sommer, B. (2018). Heuristic Modeling and 3D Stereoscopic Visualization of a Chlamydomonas reinhardtii Cell. *Journal of Integrative Bioinformatics*, *15*(2). doi: 10.1515/jib-2018-0003

Erickson, E., Wakao, S., & Niyogi, K. K. (2015, April 9). Light stress and photoprotection in Chlamydomonas reinhardtii. Retrieved from https://onlinelibrary.wiley.com/doi/full/10.1111/tpj.12825.

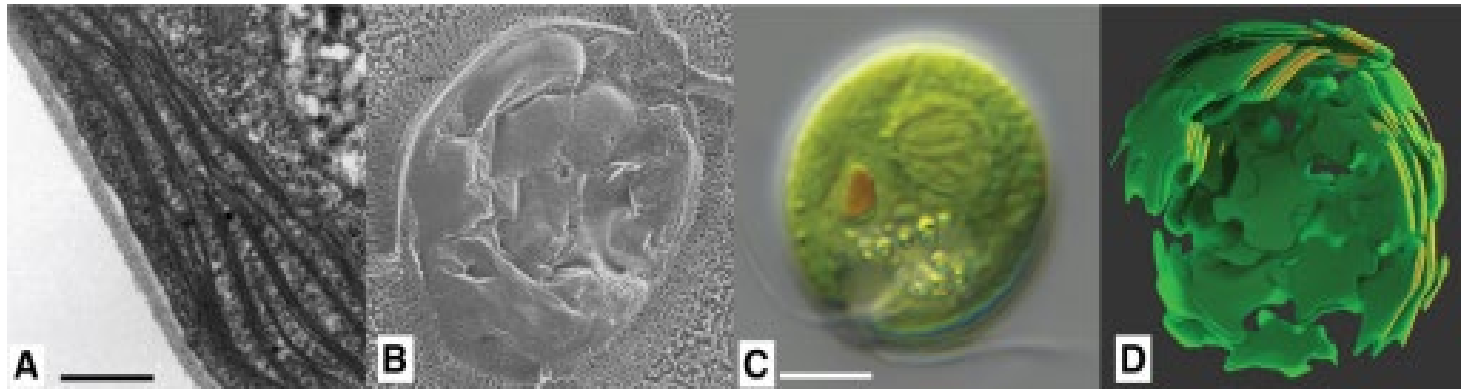


Figure 1. Chloroplast of Chlamydomonas: (A) TEM slice (scale bar 500 nm) (©2018 Trustees of Dartmouth College/Public Domain [64]). (B) Freeze fracture SEM image (×35.000, for scale bar see c) (©2014 Courtesy of Ursula Goodenough/John Heuser). (C) Light microscopy image (scale bar 5 µm) (©2016 Courtesy of Wolfgang Bettighofer). (D) Chloroplast plates rendered with final material, partly truncated, with highlighted cleaved surfaces to be compared with (A).

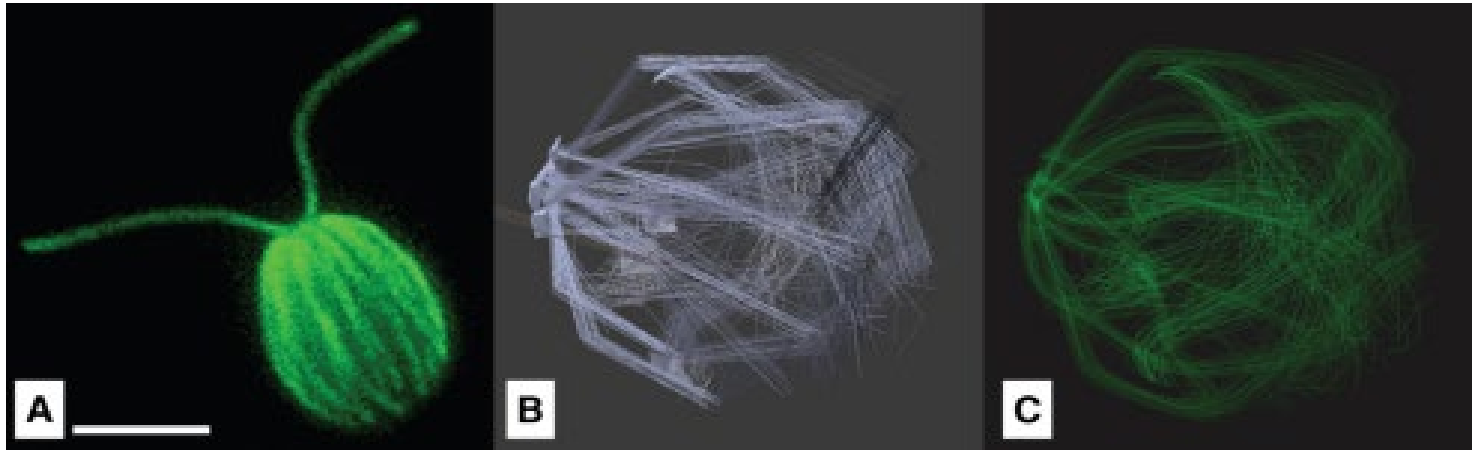


Figure 2. Microtubules filaments of the cytoskeleton: A) Chlamydomonas reinhardtii fluorescently labeled with an antibody to tyrosinated tubulin (©2018 Courtesy of Karl Johnson, procedurally as described in [69]) B) Hair Emitter with emitting planes. A) Rendered tubule model.

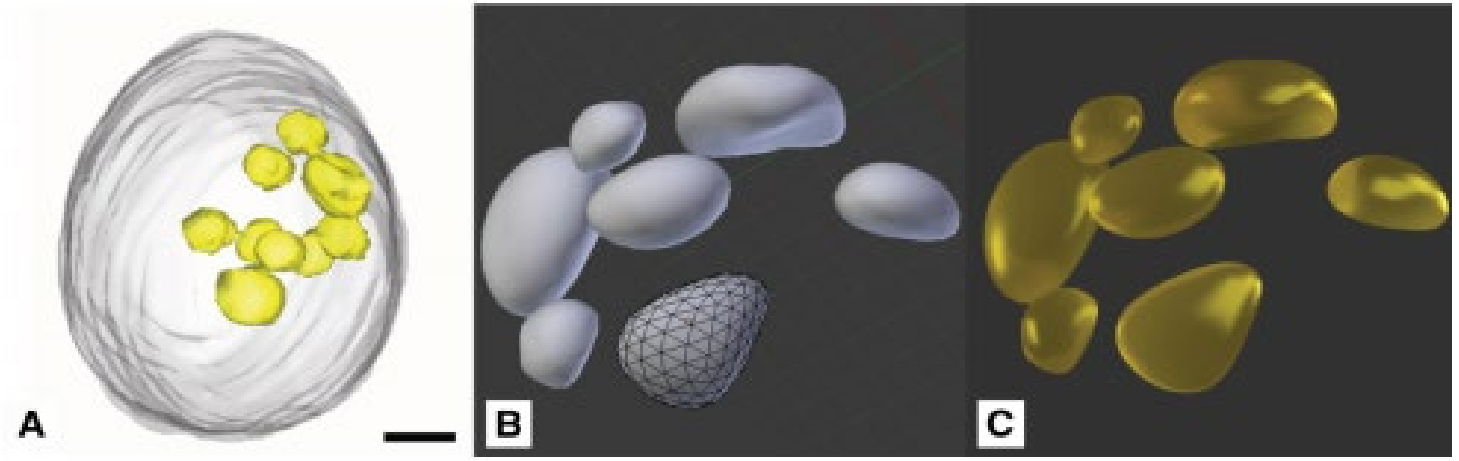


Figure 3. Lipid deposits: (A) X-ray tomography model (scale bar 1 µm) (©2012 Hummel et al., CC BY 4.0 [31]). (B) Lipid droplets modeled in Blender. (C) Rendered Lipid droplets with final material.

Biere, N., Ghaffar, M., Doebbe, A., Jäger, D., Rothe, N., Friedrich, B. M., … Sommer, B. (2018). Heuristic Modeling and 3D Stereoscopic Visualization of a Chlamydomonas reinhardtii Cell. *Journal of Integrative Bioinformatics*, *15*(2). doi: 10.1515/jib-2018-0003

Sustainable production of C. reinhardtii

Sustainable Production of *C. reinharditii* - Cell Culture Method

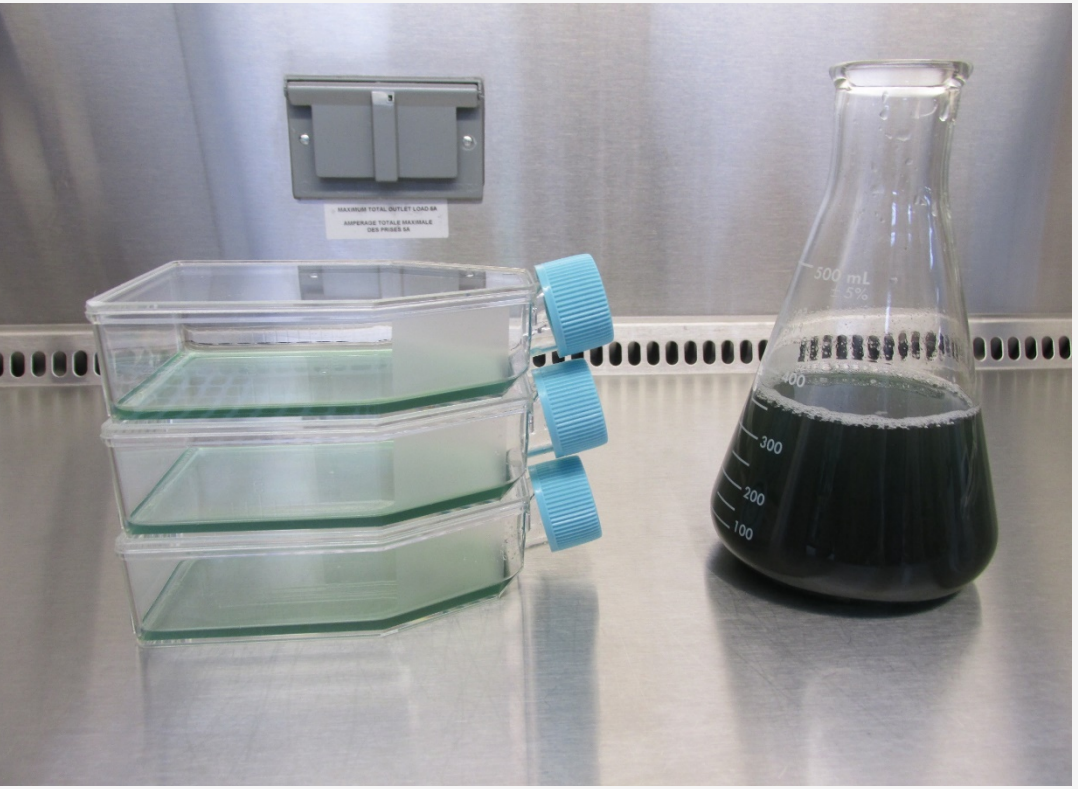


Figure 4. Microalage cultures

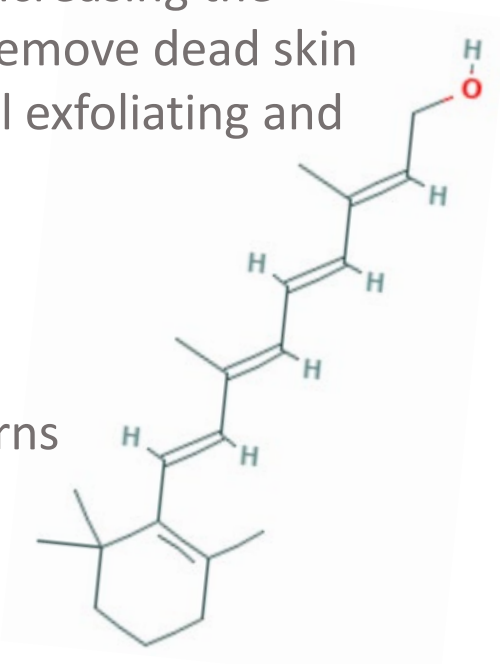
The use of plant cell culture technology allows for the production of raw materials in the most sustainable and effective manner without a negative impact on the environment and independent of location and season.

Sustainable production begins with a sample of cultured microalgae. Culture sample is transferred into a shaker flask, small aliquots of cells are transferred into T-25 flasks, where algae cells are sustainably grown under optimum conditions in cell culture lab.

The rise of ‘Clean’ cosmetics, elevates the need for sustainably sourced, natural alternatives to cult skincare favorites, including retinols and ceramides..

Retinol

Retinol is a staple anti-aging ingredient utilized to reduce fine lines and wrinkles while increasing the production of collagen. While enhancing cellular renewal, retinol exfoliates the skin to remove dead skin cells encouraging the appearance of a revitalized complexion. Retinol is not just a typical exfoliating and anti-aging ingredient.



Vitamin A derivative with pleiotropic effects

The distinctive mechanism of action sets retinol above the rest as it targets aging concerns at a cellular level. Most cosmetic ingredients address concerns on the surface of the skin, working to exfoliate and alleviate wrinkles or fine lines.

Retinol penetrates into the stratum corneum and on some occasions into the outer dermis, where it binds specifically to RAR and RXR receptors inside epidermal cells. These receptors are nuclear receptors, which act as a genetic control mechanism to alter gene expression.

Retinol influences gene regulation to transform keratinocyte cell differentiation, fibroblast proliferation, collagen synthesis, and cell adhesion in the epidermis.

Huang, P., Chandra, V., & Rastinejad, F. (2013). Retinoic Acid Actions through Mammalian Nuclear Receptors. *Chemical Reviews*, *114*(1), 233–254.
Gericke, J., Ittensohn, J., Mihály, J., Alvarez, S., Alvarez, R., Töröcsik, D., … Rühl, R. (2013, April 24). Regulation of retinoid-mediated signaling involved in skin homeostasis by RAR and RXR agonists/antagonists in mouse skin.
Dawson, M. I., & Xia, Z. (2012, January). The retinoid X receptors and their ligands.

Ceramides

Ceramides are major lipid components of the lamellar sheets in the intercellular space of the stratum corneum.

The stratum corneum is basically composed of lamellar sheets structured by sphingolipids (ceramides), free fatty acids, and cholesterol. These 3 major components make up the skin barrier.

Ceramides are waxes with higher melting point, which gives the ability to remain in solid state at room temperature. The hydrophobic nature of ceramides acts as a potent water repellant.

It is well known that ceramides play an essential role in structuring and maintaining the water permeability barrier function of the skin, therefore, strengthen and protect the skin's natural moisture.

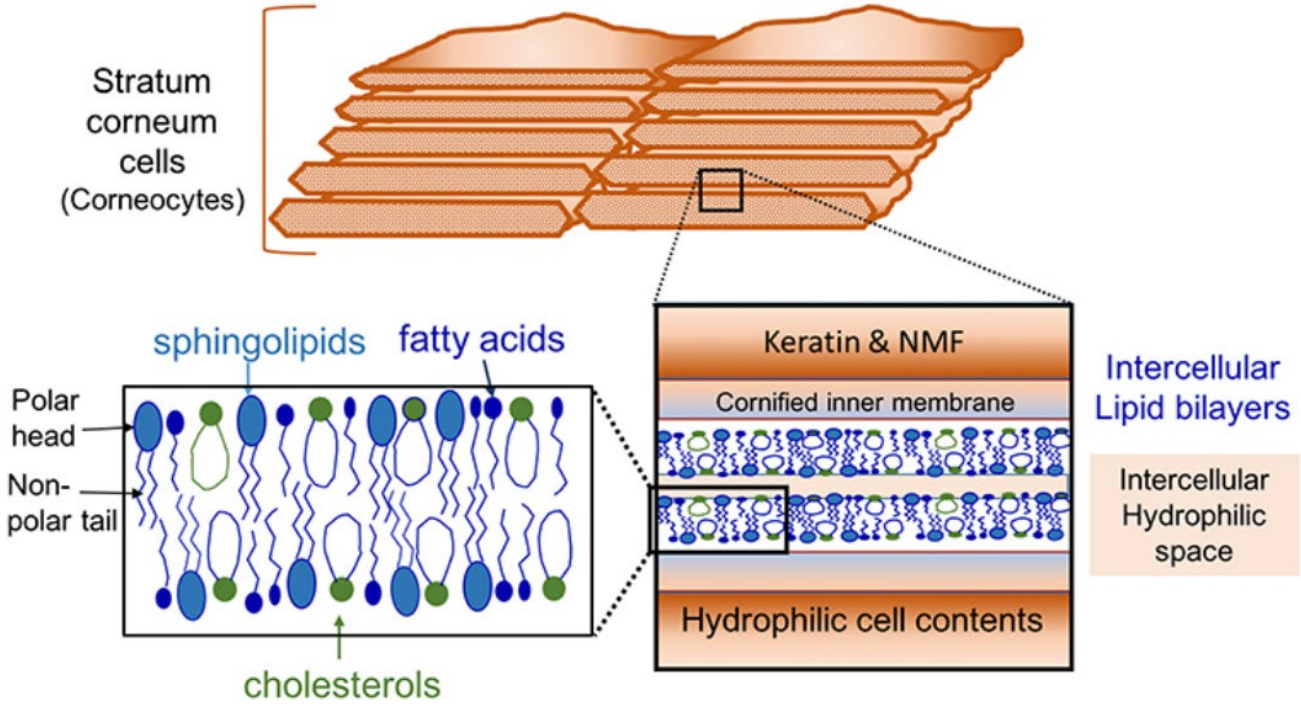


Figure 5. Structural layers of stratum corneum

Coderch, L., López, O., de la Maza, A., & Parra, J. L. (2003). Ceramides and skin function.
16-Jan-2019. (n.d.). What is the stratum corneum and its importance in skin care? Retrieved from https://www.cosmeticsbusiness.com/news/article_page/What_is_the_stratum_corneum_and_its_importance_in_skin_care/143839

Skin Genomics Assay

Skin Genomics Assay

Mediation of gene expression was evaluated in cultured keratinocytes or commercial skin equivalents per protocol.

About twenty genes, important in a variety of skin functions were evaluated.

Both the **RxR** and **PPAR gamma** gene code for nuclear regulatory factors and work together in modulating a number of other key genes involved in proliferation and inflammation.

C. reinhardtii lipids increased expression of both PPAR gamma and RxR, indicating that the use of *C. reinhardtii* lipids would likely result in increased epidermal proliferation.

DNA Microarray Analysis

RNA Isolation

RNA Concentration Assay

RNA Gel Electrophoresis

mRNA Amplification

In Vitro Transcription:
Synthesis & Purification of aRNA

Labeling & Purification of aRNA

Microarray Hybridization & Washing

Microarray Scanning & Analysis

Skin Genomics Assay – Results

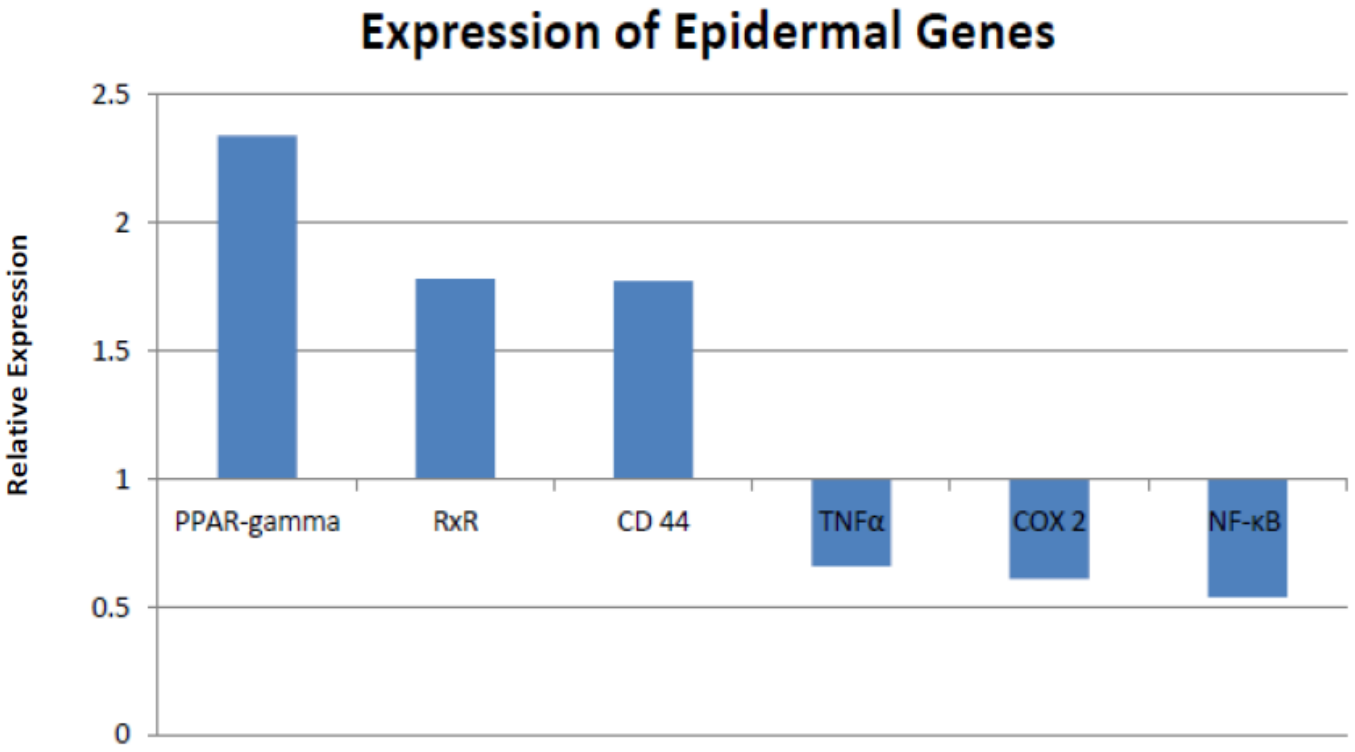


Figure 6. Expression of epidermal genes.

Three genes important in the skin inflammatory process were down-regulated; these include NF-κB, TNFα and COX 2 (cyclooxygenase).

CD 44 involved in cell adhesion and usually down regulated during differentiation was up-regulated suggestion a shift from differentiation to proliferation. All data presented herein was statistically significant

Skin Genomics Assay – Results

Mediation of gene expression was evaluated in cultured keratinocytes as per commercial protocol.

Twenty genes, important in a variety of skin functions were evaluated. Both RxR and PPAR gamma are nuclear regulatory factors and work together in modulating a number of key genes involved in proliferation and inflammation.

Chlamydomonas reinhardtii lipids increased expression of both PPAR gamma and RxR. Three genes important in the skin inflammatory process were down-regulated; these include NF-κB, TNFα and COX 2 (cyclooxygenase).

CD 44 involved in cell adhesion and usually down regulated during differentiation was upregulated suggestion a shift from differentiation to proliferation.

Summary

Bioactive algae lipids are the next generation cellular modulators similar to our cult skincare favorites, retinols and ceramides.

One of the leading raw materials, algae, is leading sustainability in various industries including biofuels, renewable energy, nutraceuticals, and food.

The rise of plant-based and natural cosmetics, prompted algae based bioactive molecules to the forefront.

C. reindharditii, a species of green microalgae naturally exhibits retinol and ceramide like properties due to its structural and physiological features which also function as the mechanism of survival and motility.

Currently, further research is required to determine the mechanism bioactive *C. reindharditii* lipids in in-vitro models in regards to skin care.

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Thank You!