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Phytoplankton: The New Frontier for Stress-Relieving Cosmetic Ingredients

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Key words

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Abstract

Phytoplankton or micro algae is a diversified, sustainable and ecological source of innovative ingredients for cosmetics. We introduce three species that help relieve the skin from various stresses and restore homeostasis: Phaeodactylum tricornutum, Porphyridium cruentum and Skeletonema costatum.

The skin suffers various stresses such as UV radiation, aging and aggressions that disrupt its normal function. Traditional cosmetic answers are found either in vegetable, biological or biotechnologically derived ingredients. A new diversified, sustainable and ecological source is now available: phytoplankton (a unicellular microscopic alga). Various phytoplankton extracts have been found to relieve skin stresses and restore skin homeostasis.

Phaeodactylum tricornutum Extract

Phaeodactylum tricornutum belongs to the Diatomophyceae order widely found in coastal waters. The lipidic fraction is obtained by a treatment with a mixture of water and alcohol under alkaline condition. The fatty acids are purified by extraction and molecular distillation. The extract offers a unique fatty acid profile. The key constituents are unsaturated fatty acid C16:1, C20:5, C22:6. *Phaeodactylum tricornutum* protects and repairs age- and UV-induced damage to proteins.

Aging and protein oxidation: Proteins are among the major targets for oxidative damage (in addition to DNA and lipids) and the build-up of potentially harmful oxidized proteins is characteristic of aging and leads to cellular dysfunction and senescence. The correlation between photo-damage and protein oxidation is also well-established,[1] because there is increasing evidence for the generation of reactive oxygen species (ROS) in skin upon UV exposure.

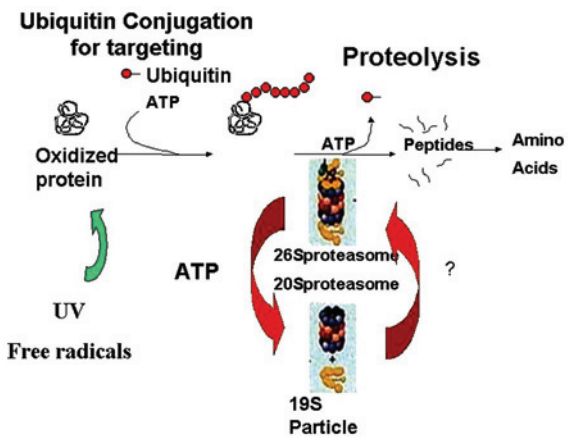

Figure 1. The ubiquitin proteasome pathway

Until now, besides using anti-oxidants and sunscreens for protecting the skin, no other protection was available. However a new line of defense can be established by modulating proteasome activity.

Protein oxidation and proteasome: Each cell contains a proteolytic system called proteasome responsible for the degradation of oxidized proteins and protein turnover. Proteasome is a multicatalytic protease complex (1.5 to 2 million Dalton) located in the cytosol and in the cell nucleus. It exhibits three distinct proteolytic activities – chymotryptic-like, tryptic-like and peptidylglutamyl peptide hydrolyzing – as it cleaves proteins on the carbonyl side of hydrophobic, basic and acidic residues. Proteolysis (Figure 1) is achieved after combining the oxidized protein with ubiquitin, which unfolds the protein before it is digested by the 26S proteasome.

The Ubiquitin Proteasome Pathway (UPP) is the cell's principal mechanism for protein catabolism and has roles in both housekeeping and the turnover of many regulatory proteins (Figure 1).

Proteolysis is central to an incredible multitude of processes including: the cell cycle; cell growth and differentiation; embryogenesis; apoptosis; signal transduction; DNA repair; regulation of transcription and DNA replication; an-

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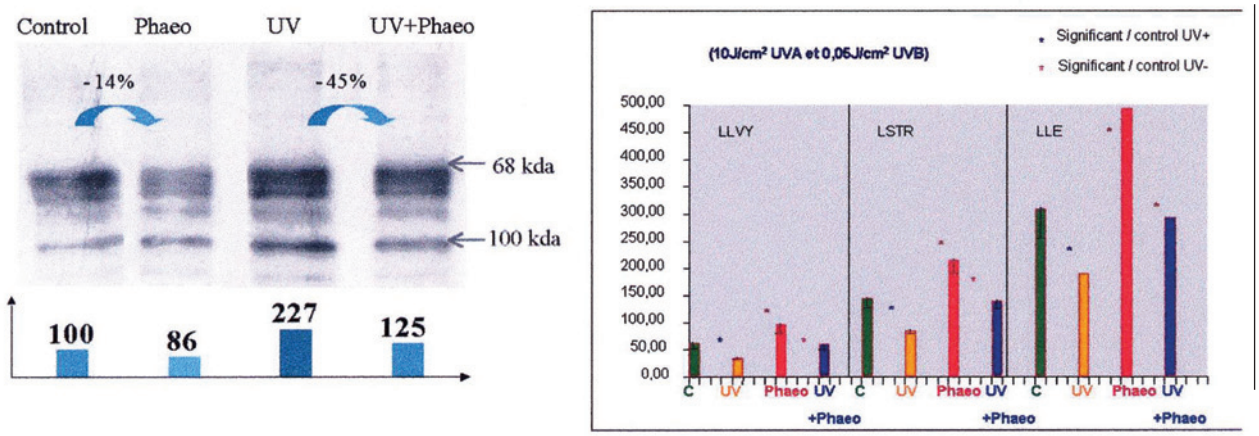

Figure 2. The repair effect of Phaeodactylum extract
a) Western blot
b) Restoration of the three protease activities of proteasomes in keratinocyte culture after UV irradiation

tigen presentation and other aspects of the immune response; the functions of the nervous system including circadian rhythms; and acquisition of memory.

Impairment of proteasome function: Proteasome function is impaired upon aging and UVA- and UVB-irradiation.[2-4] Therefore it is important to restore its activity and prevent the impairment of key enzymes for skin function such as protein kinase, catalase, p53, and proteases among others.

The *Phaeodactylum tricornutum* extract (2.5 µg/ml) prevents the increase of UV-induced oxidized proteins: 10 µg of a keratinocyte lysate is irradiated (10 J/cm² UVA + 0.05 J/cm² UVB) and treated for 7 h. The oxidized proteins are analyzed by western blot and quantified after staining of the carbonyl group (Figure 2a). A 45% reduction in the level of oxidized protein is obtained.

The mechanism of action of this repair effect is confirmed as the Phaeodactylum extract (2.5 mg/ml) restores the three protease activities of proteasome in keratinocyte culture that have been submitted to UV irradiation (Figure 2b).

Protein oxidation can be repaired: Because both chronological and photo aging increase protein damage and damage the proteosomal activity, the cells accumulate oxidized proteins that are no longer removed. The *Phaeodactylum tricornutum* extract helps to protect and repair protein oxidation and will help the skin to be purified from these wastes and reduce age- and UV-induced damages.

Furthermore, it has been proven[5] that lipofuscin – a substance composed essentially of oxidized, cross-linked proteins and a hallmark of aged non-dividing cells – present in age spots, may originate from insufficient proteasome activity. Further studies may be needed to confirm that the *Phaeodactylum tricornutum* extract may help in reducing lipofuscin content in age spots.

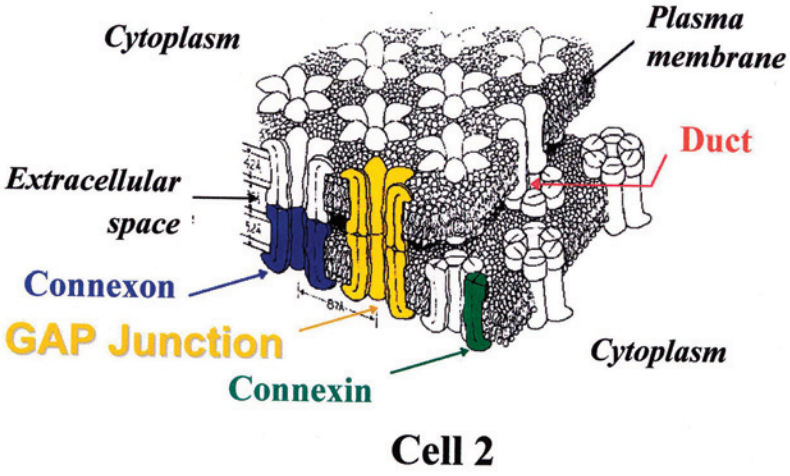

Figure 3. Gap junctions between neighboring cells

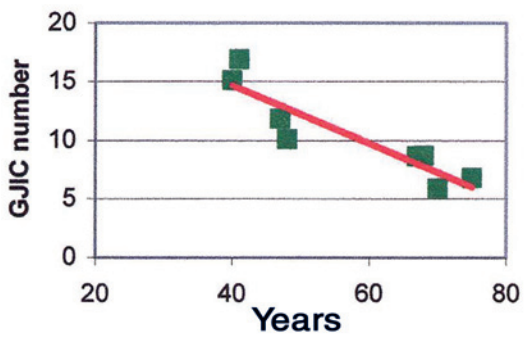

Figure 4. Aging (in years) disrupts the number of gap junctions (GJIC) in normal human keratinocytes

in particular on the quality of the communication between cells through gap junctions.

Gap junctions are areas of the cell membranes that connect neighboring cells (Figure 3). These organized protein channels allow ions and small molecules to cross between the connected cells in a passive fashion. The "communicating" cells equilibrate all of their critical regulatory ions and small molecules (Ca++, C-AMP, glutathione). These protein channels consist of two "hemi-channels" or connexons that consist of six proteins called connexins. Connexin 43 is mostly expressed by keratinocytes.[6]

Physiological functions of gap junctions: The fundamental function of two or more cells coupled by gap junctions is to communicate through chemical signals. The major physiological role of gap junctions is to synchronize "metabolic" or electronic signals between cells (regulatory ions and small molecules, e.g. Ca++, C-AMP). Cells have four basic functions: proliferate, differentiate, apoptose or die by programmed cell death, and adaptively respond if it is already terminally differentiated. In a multi-cellular organism, a delicate coordination or orchestration of these four cellular functions must occur. So in the epidermis, growth, differentiation, apoptosis and homeostatic control of differentiated cell function must occur in a single space and this is done by coupling the keratinocytes through their gap junctions.[7,8]

Aging disrupts gap junctions: The number of gap junctions in normal human keratinocytes declines according to the age of donors (Figure 4). However, we observed a dose dependent increase in the number of gap junctions following micro-injections of confluent normal human keratinocytes (NHK) (Figure 5).

Various authors[9,10] have also proven that UV radiation disrupts gap junctional communication in human keratinocytes and contributes to skin photo-aging.

Skeletonema costatum lipidic extract restores gap junctional communication: When keratinocytes from a 63-year-old donor are treated with 2.5 µg/ml of *Skeletonema costatum* lipidic extract, the number of gap junctions (GJIC) is increased by 78% and reaches the number of GAP junctions found in keratinocytes from younger donors. By this effect, a *Skeletonema costatum* lipidic extract helps to restore and maintain epidermal homeostasis and cellular harmony.

Skeletonema costatum Lipidic Extract

Skeletonema costatum is a monocellular alga from the Diatomophyceae order. It is widely found in the coastal waters. The micro algae are treated with a mixture of water and alcohol under alkaline conditions. The fatty acids are purified by extraction and molecular distillation. The extract offers a unique fatty acid profile. The key constituents are polyunsaturated fatty acids: C16:3, C18:4, C20:5 and C22:6.

Cell communication and skin homeostasis: The loss in homeostatic capacity of the aging organism depends

Porphyridium cruentum Extract

During its growth, *Porphyridium cruentum*, a red micro alga, synthesizes high molecular weight polysaccharides that create a protective coating around the cell membrane. Part of these polysaccharides will dissolve in seawater and

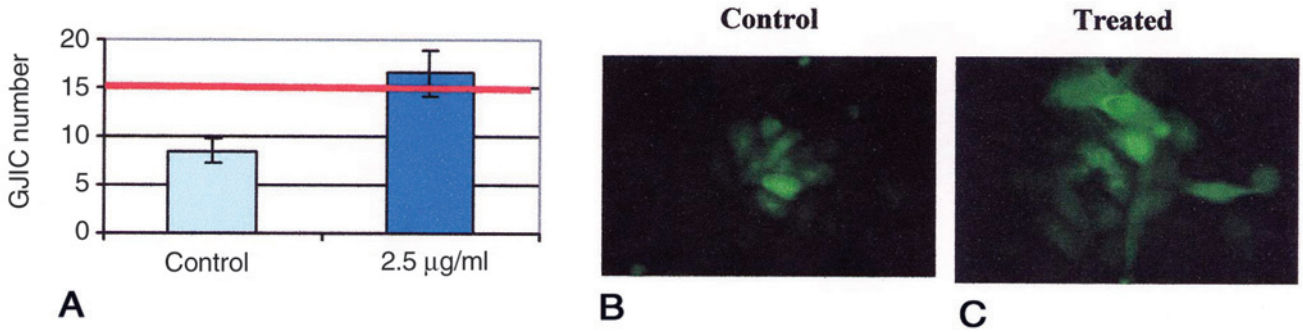

Figure 5. Effect of micro-injected Skeletonema costatum lipidic extract on gap junctional communication
a) Restoration of number of gap junctions (GJIC) in older donors following treatment versus untreated control, and comparison at 15 to the number of GJIC in young donors (correlation: r=0.91)
b) Control
c) Treated

increase the viscosity locally. This is really a unique self-protection mechanism!

Porphyridium cruentum extract is obtained from red micro algae grown in Brittany in marine spring water whose unique mineral composition boosts its duplication. The extract is therefore composed of a solution of polysaccharides in minerals.

Special polysaccharide structure: *Porphyridium cruentum* excretes a high molecular weight (around 4,000,000 D) sulfated polymer, similar to human glycosaminoglycans. Its major components[11] are listed in Table 1. It contains three disaccharides and two uronic acids. The disaccharides are 3-O(alpha-D-glucopyranosyluronic acid)-L-galactose, 3-O-(2-O-methyl-alpha-D-glucopyranosyluronic acid)-D-galactose, and 3-O-(2-O-methyl-alpha-D-glucopyranosyluronic

acid)-D-glucose. A polyanion of high molecular weight contains D- and L-galactose, xylose, D-glucose, D-glucuronic acid and 2-O-methyl-D-glucuronic acid, and sulfate in molar ratio (relative to D-glucose) of 2.12:2.42:1.00:1.22:2.61.

The *Porphyridium cruentum* extract, obtained from a marine spring water, is rich is various beneficial trace elements – manganese, silicium and zinc – at levels ten times higher than in normal seawater. These trace elements are essential cofactors used by skin enzymes for its normal function.

Immuno-modulating properties: The polysaccharide of *Porphyridium cruentum* significantly increases the number of cells present in the peritoneal exudate. It also increases the activity of lysosomal acid phosphatase enzyme. These facts suggest a possible stimulation of the metabolic and functional action of the immune system.[12] Topically, the polysaccharide of *Porphyridium cruentum* limits irritant-induced cutaneous erythema, probably by inhibiting circulating immune cell recruitment.[13]

Table 1. Major components of the polysaccharide excreted by *Porphyridium cruentum*

Hexoses (galactose / glucose)	36.0%
Pentoses (xylose)	30.0%
Glucuronic acid	8.0%
Sulfate	9.0%
Amino acids (xylose linked)	3.8%

Furthermore, the polysaccharide of *Porphyridium cruentum* exhibited strong antiviral activity against herpes simplex virus types 1 and 2 both in vitro and in vivo, through a strong interaction between the polysaccharide and the virus.[14]

These activities suggest anti-adhesion properties similar to chondroitin sulfate, a well-known modulator of the immune responses and activator for wound healing. Further work will be needed to fully explore this field of research.

Moisturization and barrier repair: Due to its high molecular weight polysaccharide (higher than hyaluronic acid) and to its unique mineral composition, the *Porphyridium cruentum* extract offers excellent moisturizing properties in two ways: through forming a non-occlusive film on the skin surface and by enabling the skin to synthesize the epidermal lipids necessary for barrier recovery. Figure 6 shows the results of tests on a keratinocyte culture. At 100 µg/ml, the *Porphyridium cruentum* extract increases in average the barrier lipids by 45%.

Barrier repair is essential for preventing transepidermal water loss and maintaining skin homeostasis.[15] Allowing the skin to synthesize its own requirements represents an elegant way to retain its normal functioning even under stressed conditions.

Skeletonema costatum Peptidic Extract

During an aggression (from UV or other sources), the skin defends itself by recruiting immune cells. Keratinocytes release cytokines (such as IL1a and TNFa) and chemokines (IL8). They express adhesion molecules (selectins, ICAM 1s) that allow the adhesion of leukocytes on the endothelial cell of micro-capillaries and their release in the inflamed tissue that leads to erythema. Inhibiting leukocyte adhesion prevents the endothelial cells from acting as inflammation amplifiers.

Human leukocyte adhesion on endothelial cells from the dermal capillary is stimulated by a UV-irradiated NHK culture medium. Human keratinocytes (from plastic surgery) were cultured in the presence of tested molecules for 48 to 72 hours after UVB irradiation (10 mJ/cm^2) to condition the media.Then, the leukocyte (human T cell line CEMT4) adhesion and endothelial cell (immortalized human micro vascular HSMEC 7,8) adhesion molecule expression were assayed on keratinocyte-conditioned medium-treated endothelial cells.

To evaluate leukocyte adhesion, FITC-labeled leukocytes were overlaid onto PKH26GL-labeled endothelial cell monolayer in five to one endothelial cell ratio and were allowed to adhere for 20 min at 4°C, under static conditions. After removing non-adhering cells, endothelial cells and adhering cells were lysed and analyzed by fluorescence. IL 8 were assayed by ELISA kits.

Adhesion molecules expression of ICAM 1s was analyzed by flow cytometry after incubation with FITC antibodies.

Skeletonema costatum peptidic extract (SKCP) was applied on keratinocyte cultures at 40 µg/ml.It inhibits IL8,ICAM 1s and leukocyte adhesion by 42%, 69% and 37%, respectively.

Modulation of pro-inflammatory condition and erythema prevention are traditionally performed by inhibiting the cytokines that enhance the PLA2 activity, release arachidonic acid that is metabolized by the lipoxygenase pathway to produce leukotrienes, or by the cyclooxygenase pathway to produce prostaglandins and thromboxanes.Inhibiting leukocyte adhesion will modulate another key inflammation mechanism. Thus, *Skeletonema costatum* peptidic extract offers a new promising approach for relieving irritated skin.

Another important field of cosmetics dealing with inflammation is the acneic skin. It has been proven that the *Propionibacterium acnes* has a key role in acne-related inflammation because it induces IL-8 production.[16] Also, acne-related inflammation is mediated by CD4+T cells with a high level of ICAM-1s expression and leukocyte infiltration.[17,18] Because *Skeletonema costatum* peptidic extract limits those

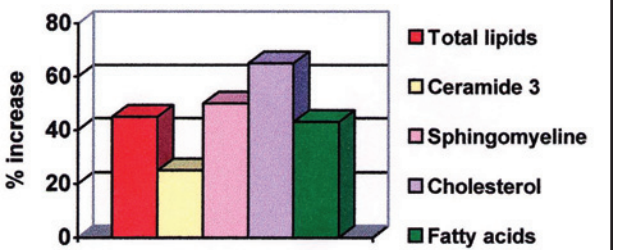

Figure 6. Average increase in epidermal barrier lipids following application of 100 µg/ml of Porphyridium cruentum extract

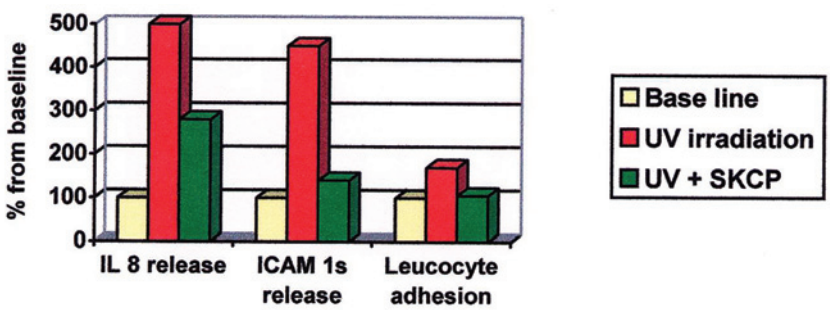

Figure 7. Anti-inflammatory effect of Skeletonema costatum peptidic extract applied to keratinic cultures at 40 µg/ml

phenomena, it will help improve the comfort of acneic skin.

New Perspectives for Relieving Skin Stress

By helping repair oxidized proteins, restore cell communication, promote barrier repair and limit the consequences of inflammation, various phytoplankton extracts will limit the consequence of many daily stresses and restore skin homeostasis.

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