

Marine actives: taking care in way forward

Codif, which offers a range of active ingredients extracted from coastal plants and algae, will always have marine extracts at the heart of its business.

Up until now, the philosophy of the company could be summed up in the words "Observe", "Be inspired", and "Reveal". Now there is an additional word: "Preserve".

The sea is not an inexhaustible source of beauty. Highly technical marine ingredients must be harvested in strict compliance with a controlled management programme for natural resources.

Observe: samphire is associated with a scientific discovery

The first aim of the Codif research laboratories is to observe the sophisticated adaptive systems developed by algae or coastal plants to survive in specific conditions. This first observational stage often generates new perspectives for cosmetic applications as with samphire and the discovery of Aquaporin 8 in the epidermis.

Samphire is a perennial coastal plant that has developed extraordinary adaptive capacities linked to the presence of water and ammonium ion transporters known as gamma-TIP (Tonoplast Intrinsic Protein) which play a fundamental role in protection against dehydration and the high salt levels to which it is subjected on a daily basis.¹ The gamma-TIP found in *Salicornia herbacea* has been extensively studied: it is a water channel, involved in salt tolerance, whose sequence presents a strong similarity with that of human Aquaporin 8 (AQP8).²

AQP8, which up to now was less well known than its homologue AQP3, is an ammonium ion transporter,³ the latter being an essential precursor for the synthesis of urea.

In 2007, Codif demonstrated their presence, for the first time, in human epidermis. The cutaneous expression is therefore a major discovery and has a central role in urea metabolism and thus the moisturising mechanisms of the skin.

The company took inspiration from the properties of samphire and developed a

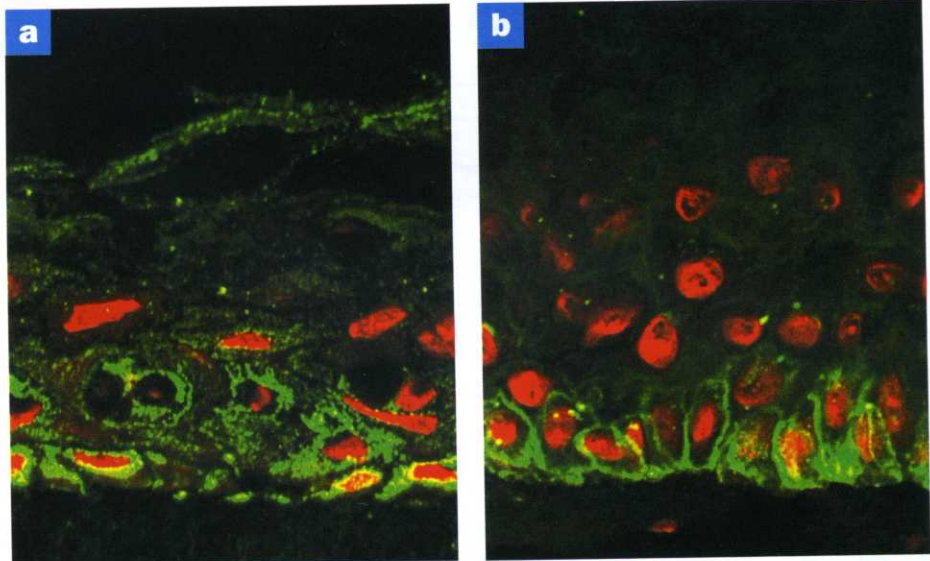


Figure 1: Immunofluorescence view, using a confocal microscope, of AQP8 (a) and AQP3 (b) in reconstituted epidermises – SkinEthic (24 hours of treatment) (magnification x120). The aquaporins are stained green (Alexa 488) and the nuclei red (propidium iodide).

samphire oil (Hydrasalinol) capable of stimulating the synthesis of AQP8 in the epidermis, and increasing the urea content of the NMF.

The mini-chip technique was used to demonstrate the expression of AQP8 mRNAs in normal cultured human epidermal cells, and in reconstituted human epidermises.

The presence of the AQP8 protein was demonstrated in the epidermis of human skin and in reconstituted cultured human epidermises, using immunohistochemical imaging with a specific AQP8 antibody. AQP8 is expressed in the basal layer of cell membranes and in the cytoplasm of supra-basal cells. A more precise immuno-localisation of AQP8 was obtained in epidermises using confocal microscopy (Fig. 1). It has also been detected in cultured keratinocytes.

Hydrasalinol, at a concentration of 1%, has been shown to stimulate the synthesis of AQP8 by 198% and that of AQP3 by 43%. These results suggest an enrichment of the urea content of the NMF, but also an optimisation of water within the various layers of the epidermis, via the stimulation of AQP3 Aquaporins.

Preserve: an exclusive nursery and algal culture programme for *Dictyopterus membranacea*

Brittany has 700 km of coastline bordered by waters of a very high quality. It is the richest region in the world in terms of algal biodiversity, and is also the biggest European reservoir. It is therefore essential to take this richness and diversity into account during the development of a cosmetic extract. The use of algal cultures alleviates the difficulties of harvesting certain algae that can only be collected by divers, and also increases the access to biodiversity without exhausting natural reserves.

With this in mind, Codif has developed an exclusive algal culture programme on a 70 hectare marine leasehold site in a protected zone at the mouth of the Rance river.

The culture of *Dictyopterus membranacea* is a prime example of work conducted. *Dictyopterus membranacea* is a brown alga from the European Atlantic coast from the Dictyoptal family. The difficulty of harvesting by diving, its dispersion in the Channel and the protection of the species in the Mediterranean, have prompted researchers

at the company to develop culture methods at sea to provide a regular supply without depleting the natural resources. The algae, which are initially harvested in the "wild", are placed in hatcheries, where they emit spores that ensure their durability (Fig. 2a). The germination stage gives rise to small algae that are then transferred to the open sea in culture zones on rope lines (Fig. 2b). One year after the nursery stage, the algae are harvested for extraction.

The aromatic fragrance of *Dictyopteris membranacea* is very characteristic and gives certain bays (Hawaii) a marine odour due to the presence of dictyoptere-type pheromones. An oily extract of *Dictyopteris* has been developed, exploiting the characteristics of dictyopterenes to produce a volumising effect on the lips and breasts.

Be inspired: *Laminaria digitata*

At the beginning of the decade, several studies demonstrated the beneficial effects of beta-glucans for human health, notably through the presence of numerous polysaccharides with anti-inflammatory properties. The company decided to develop a marine extract capable of transferring the beneficial properties of beta-glucans into cosmetics.

Among the algae that were recorded and observed regularly, *Laminaria digitata* was rapidly singled-out as an interesting candidate. This macro-alga from the coast of Brittany covers itself in mucilage, rich in alginates, whose composition is relatively similar to that of the beta-glucans.

Incapable of controlling the accumulation and degradation of these polysaccharides by itself, the alga has developed a symbiotic lifestyle with a marine bacterium that produces alginate lyase. This symbiotic lifestyle is characteristic of a sophisticated defence mechanism. When the alga is attacked by pathogenic bacteria, the symbiotic bacteria trigger an overproduction of alginate lyase. The component alginates of the algal mucilage are then digested into oligoalginates. These oligoalginates then participate in the



Figure 2: a) Spores of *Dictyopteris membranacea* in a nursery. b) Adult plants of *Dictyopteris membranacea* after one year of culture at sea on rope lines.

alga's defence system by stimulating the synthesis of lectin, a protein involved in the development of the immune system. The study of this perfected symbiotic defence system reveals the immunoprotective properties of these oligoalginates.

Researchers at the company took inspiration from these symbiotic defence mechanisms to reconstitute this oligoalginate with immunoprotective properties.

The *in vitro* control of the growth of the symbiotic bacteria enables the production and purification of alginate lyase. The latter is then brought into contact with membranes of *Laminaria digitata* and the digestion process is initiated.

The first oligoalginate obtained has an average molecular weight (around 25 kDa) with interesting film-forming and protective properties. This oligoalginate was subsequently named Phycosaccharide AP (Anti-Pollution). It has a specific affinity for the skin, forming a film that protects it against environmental pollution (Fig. 3). This property is also exploited in a moisturiser, Pheohydrane, used to limit water loss.

With the ongoing aim of obtaining an immunoprotective oligoalginate, the contact time between the algal membranes and the bacterial enzyme was increased to perfect the digestion and obtain an

oligoalginate with a lower molecular weight. To obtain a molecule with a degree of polymerisation of 10 (around 3 kDa), and non-viscosity, the enzymatic digestion was combined with acid hydrolysis. The resulting oligoalginate was then tested for its immunoprotective properties and the first *in vitro* tests demonstrated the inhibition of the principal inflammatory mediators such as PGE₂, interleukin 1 alpha, histamine, etc., while protecting immune cells such as the Langerhans cells.

This oligoalginate also presents the particularity of having free carboxyl groups, indicating the possibility of forming complexes with bivalent cations to produce additional properties. A new active ingredient, Phycosaccharide AC, was developed by chelation of the oligoalginate to Zn²⁺ ions. It has anti-inflammatory properties and limits the growth of microorganisms, making it suitable for anti-acne applications. Finally, Thalitan is issued from the chelation of the oligoalginate with Mg²⁺ and Mn²⁺ ions for anti-ageing properties.

Very recently, researchers at the company demonstrated the capacities of Phycosaccharide AI to optimise the recruitment and mobilisation of adult epidermal stem cells, essentially due to the specific linkage of guluronic and mannuronic residues of which it is composed.

Today, the phycosaccharides constitute a range of biotechnological marine agents that are ideal for protecting and soothing sensitive and problematic skin.

Reveal: a concentrate of kombu rejuvenates adult stem cells of the dermis

Revealing the beauty of the skin is about reawakening its youth and that of its cells. As such, stem cells present interesting perspectives.

Ageing of the skin and the slowing of its regeneration are due to poor mobilisation

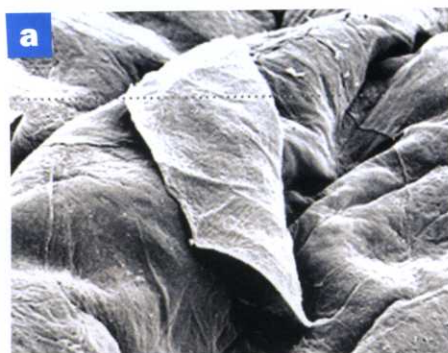


Figure 3: Observation of the affinity of an average molecular weight oligoalginate (Phycosaccharide AP) for the skin and its film-forming effect. a) Untreated skin. b) Skin treated with Phycosaccharide AP.

of adult stem cells, and/or to a reduction in the strength of signals for renewal that stimulate the adult stem cells.⁴

In both cases, these dysfunctions are due to the ageing of the adult stem cells. Over time, their capacities for renewal decrease, and tissue regeneration slows.⁴

While the first studies concerning stem cells involved stimulating their multiplication, recent studies show that the exhaustion of stocks of adult stem cells in the skin may occur following forced proliferative stress. Molecular mechanisms therefore seem to be capable of limiting the number of divisions of stem cells.^{5,6} The capacities for renewal of adult stem cells of the dermis and epidermis are therefore not infinite.

Rather than intervene in the processes of multiplication or proliferation of stem cells, the company has developed a new approach. It is studying the revitalisation of ageing adult stem cells to maintain sufficient numbers of adult dermal stem cells, protect them from ageing, and renew their activity to strengthen the extracellular matrix, and make the skin look younger.

The company has developed a concentrate of kombu, known as Phycojuvenine, and has demonstrated a significant revitalisation of adult stem cells in mature skin – cellular vitality is restored to that of young skin: 95% vs 99% (Fig. 4). It then exerts a protective action with respect to cellular ageing factors, notably UVB. While rejuvenating the adult stem cells, and protecting them from ageing, Phycojuvenine then exerts a rejuvenating effect on the elderly

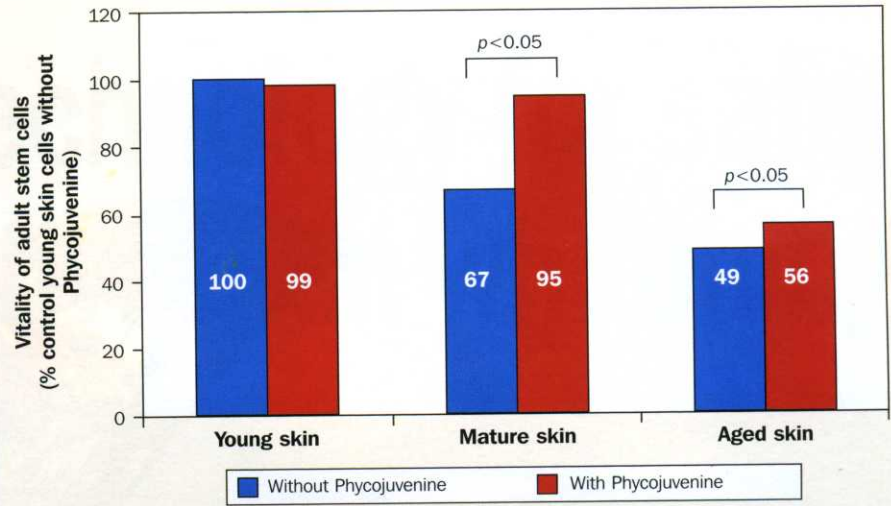


Figure 4: Effect of a concentrate of kombu (Phycojuvenine) on the revitalisation of adult stem cells of the dermis.

fibroblasts that can then synthesise similar quantities of collagen to those of young fibroblasts: 137% vs 150%.

Finally, the agent significantly decreases the depth of crow's foot wrinkles (-6.2% on average and up to -15.6%) with a smoothing effect and a decrease in mean roughness of up to -29.6%. **PC**

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