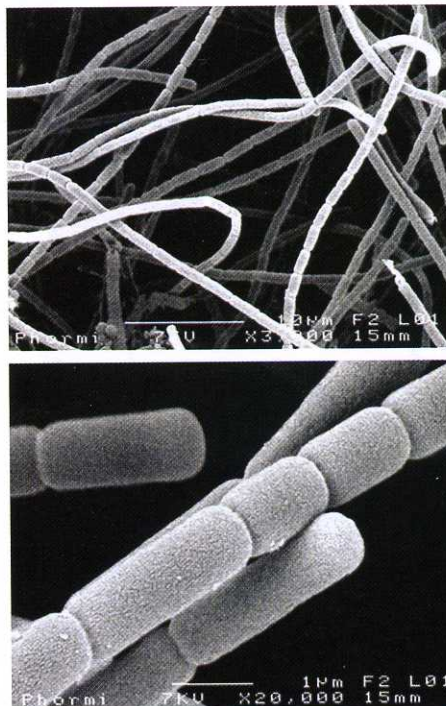


# New solution for brighter and younger skin

Skin senescence is visually characterised by a loss of freshness, a loss of radiance and a decrease in complexion homogeneity. Skin senescence is internally caused by a loss of regeneration capacities, a loss of defence capacities and an increase in cellular senescence. Fighting against skin senescence necessitated finding the most sophisticated photo-protection mechanism.

The skin is the constant target of active forms of oxygen generated by UV radiation exposure. In this way, the skin needs to reinforce antioxidant defence mechanisms to protect itself against cell damage. The defence strategies include the thioredoxin reductase/thioredoxin system.<sup>1</sup> Thioredoxin is one of the major constituents of the thiol reduction system: it has a dithiol-disulfide oxido-reductase activity.<sup>2</sup> The thiol group gives thioredoxin antioxidant properties by protecting the mitochondrial membrane against oxidation. Thioredoxin protects cells against the cytotoxicity produced by free radicals (hydrogen peroxide)<sup>3</sup> and by some molecules produced during inflammation (TNF alpha). Thioredoxin plays multiple roles in cell processes such as cell proliferation, differentiation and apoptosis.<sup>4</sup> It favours bonding with DNA transcription factors (e.g. NF-kappa B<sup>5</sup>, AP-1, p53 and PEB2<sup>6</sup>). Thioredoxin has an important role in the regeneration of the reduced form of ascorbic acid (vitamin C), which has an antioxidant power when it is in its reduced form<sup>7</sup> and which is a collagen stimulator. There are two types of thioredoxin which each occur in different areas:



**Figure 1:** Observation by scanning electron microscopy of cyanobacteria *Phormidium persicinum*.

TX1 is cytosolic and TX2 is mitochondrial.

Thioredoxins are the best preserved and most elaborate photo-protection mechanism ever created. They are widely distributed and highly conserved. We can find them extensively in humans, animals, plants and marine bacteria (including primitive bacteria which are responsible for the development of life on Earth).<sup>8</sup>

## ABSTRACT

Phormiskin Bioprotech G is a skin rejuvenator. This biotechnological concentrate of original life stimulates the synthesis of thioredoxin in the dermal and epidermal cells, delays skin senescence and improves skin radiance, freshness and homogeneity.

*Phormidium persicinum*, from the family of blue micro-algae, appeared 3.8 billion years ago and belongs to the species that contributed to the build up of Earth's atmosphere and the development of life on Earth. It is organised in mucilage-producing colonies that generate geological formations called "stromatoliths" (from the Greek "stroma", for carpet and "lithos", stone). To fight against stressful environmental conditions and to ensure its longevity through billions of years, *Phormidium persicinum* has synthesised a thioredoxin system very similar to those of humans.

The growth of stromatolite is very slow: 0.4 mm per year and the process is now well understood. So, the team at Codif International decided more than 10 years ago to find a source of *Phormidium persicinum*. As it was impossible to extract it from stromatolites (due to protection and very slow growth), the team decided to contact microbial mat specialists. They found a place where *Phormidium persicinum* was isolated in 1954 by Giovanni Provasoli at Woods Hole in Massachusetts. He isolated it from microbial mats in a place where the first steps of stromatolites formations were observed: Sippewissett Salt Marsh in Woods Hole. This discovery and the pure blue algae (cyanobacteria) were carefully preserved in UTEX: the culture collection of algae in the University of Texas. The team bought a sample from the bank of algae (Fig. 1) and started to grow it in bioreactors (Fig. 2): we developed cutting-edge biotechnological tools to extract *Phormidium persicinum* from a natural environment without altering its remarkable



**Figure 2:** Different steps of culture of *Phormidium persicinum* from laboratory to industrial scale.

capacities that have ensured its longevity and durability for billions of years. A concentrate of original life was therefore obtained, to stimulate thioredoxin synthesis in human skin and delay skin senescence.

This was the start of an incredible discovery: the reason why *Phormidium* went through the ages without changing thanks to the production of thioredoxin, one of the most ancient enzymes, the one that we find in every human cell, in particular, skin.

An extract of *Phormidium persicinum* was developed, diluted to 50% in glycerin, without preservative, and called Phormiskin Bioprotech G. This paper presents the main results obtained with this extract on human skin (*in vitro*) and volunteers (*in vivo*).

**Results**

**In vitro test: stimulating thioredoxins expression**

Thanks to Western blot analysis, it was discovered that Phormiskin Bioprotech G (now referred to as “the novel concentrate”), used from 0.5% stimulates the expression of TX1 and TX2 in the dermis and the epidermis (Fig. 3).

This action favours the reinforcement of the skin’s internal defences and the reactivation of the repair and cell regeneration processes.

**In vitro test: protecting cellular DNA**

Pyrimidine dimers are characteristic structures found in DNA damaged by exposure to UV light. They were detected by immunohistochemistry on histological sections of irradiated human skin explants treated or not with the novel concentrate.

The novel concentrate, used from 2%, has a protective effect towards UV: applied 24 hours prior to UV irradiation, it reduces the appearance of pyrimidine dimers, detected by immunohistochemistry (Fig. 4).

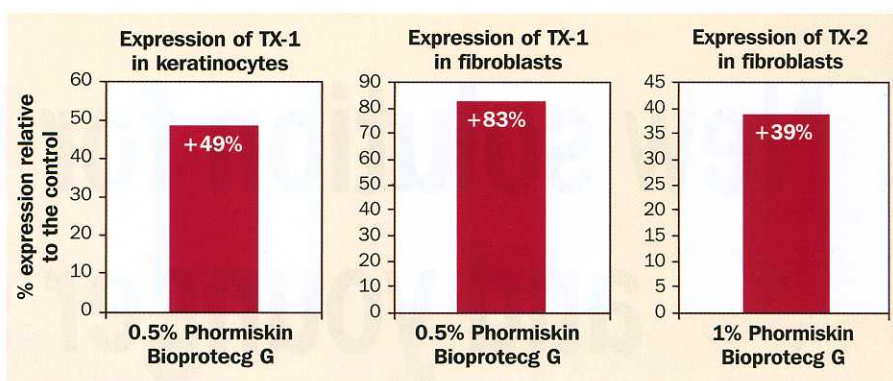
By stimulating skin thioredoxin 1 and 2 syntheses, the novel concentrate inhibits DNA lesions that lead to skin ageing.

**In vitro test: inhibiting cellular senescence**

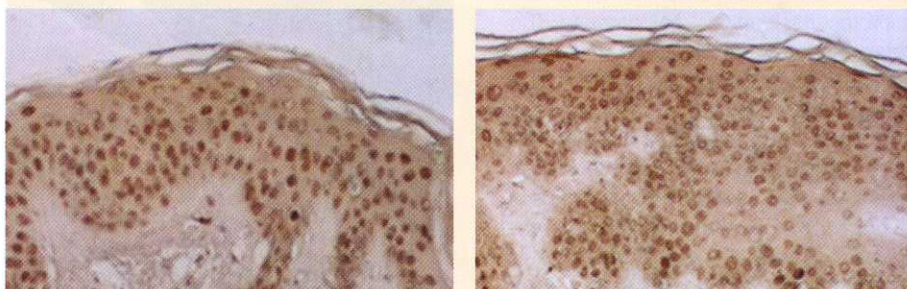
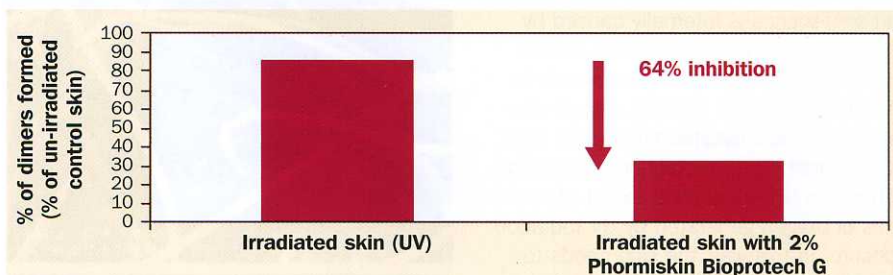
Caspase-3 is a characteristic enzyme of the cell death phenomenon called apoptosis, which occurs when cell damage has become too extensive to repair. Exposure to UVA and UVB caused an increase in caspase-3.

The novel concentrate has a protective effect towards UV: applied 24 hours prior to UV irradiation, it reduces the activation of caspase-3, detected by immunohistochemistry, in a dose-dependent manner (Fig.5).

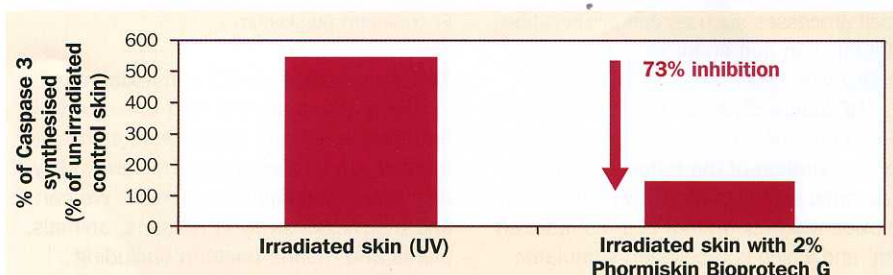
By stimulating skin thioredoxin 1 and 2 syntheses, the novel concentrate inhibits the cellular alteration responsible for



**Figure 3:** Detection of TX1 in proteins extracted from human keratinocytes and fibroblasts and detection of TX2 in proteins extracted from human fibroblasts, cultured in the presence of Phormiskin Bioprotech G (Quantification of western blot bands).



**Figure 4:** Detection and quantification of pyrimidine dimers on histological sections of irradiated human skin explants – treated, or not treated, with Phormiskin Bioprotech G.



**Figure 5:** Detection and quantification of Caspase-3 on histological sections of irradiated human skin explants – treated, or not treated, with Phormiskin Bioprotech G.

caspase-3 activation and apoptosis, and by this way, reduces cellular senescence.

**In vitro test: inhibiting the formation of sunburn cells**

Sunburn cells (shown by the yellow arrows in Fig. 6) are keratinocytes in cell death and have a specific morphological structure, resulting in important skin damage essentially 24 hours after the UV exposure, in supra-basal layers of irradiated explants. Exposure to UVA and UVB caused a dramatic increase in their number.

The novel concentrate, used from 2%, has a protective effect towards UV: applied 24 hours prior to UV irradiation, it reduces the appearance of sunburn cells, detected by immunohistochemistry.

The novel concentrate provides the skin with total protection against the formation of sunburn cells, the final signs of severe skin damage leading to a premature senescence of cells and skin.

**In vitro test: inhibiting TNF- $\alpha$  production**

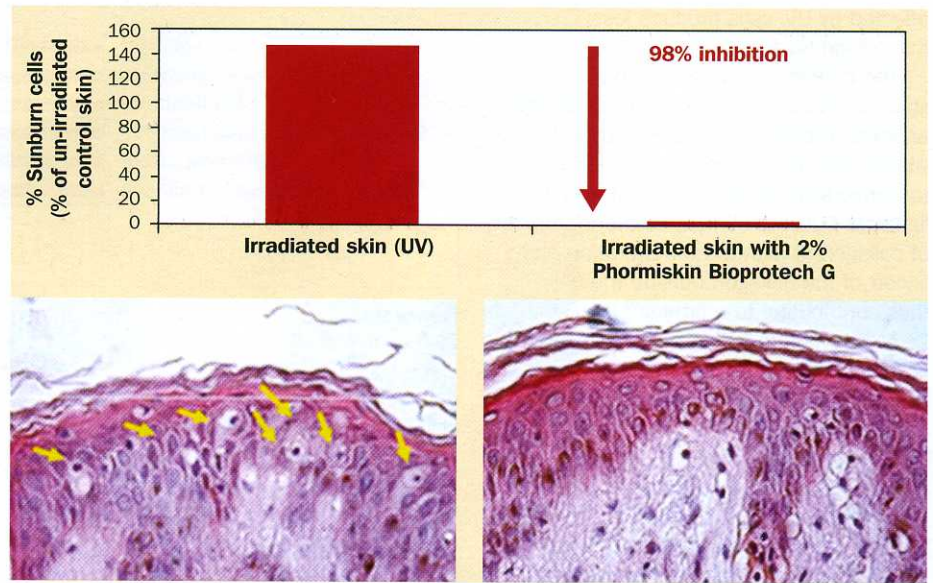
The protective activity of the novel concentrate was evaluated on human skin explants subjected to UVB irradiations known to induce damages like apoptotic cells. The parameter was the level of the cytokine TNF- $\alpha$  measured in the supernatants.

UVB irradiations induced an important increase of TNF- $\alpha$  production by human skin explants rather than the novel concentrate added at 2% to a base cream protects the explants from UVB irradiation: it decreases the production of TNF- $\alpha$  by 52% (Fig. 7).

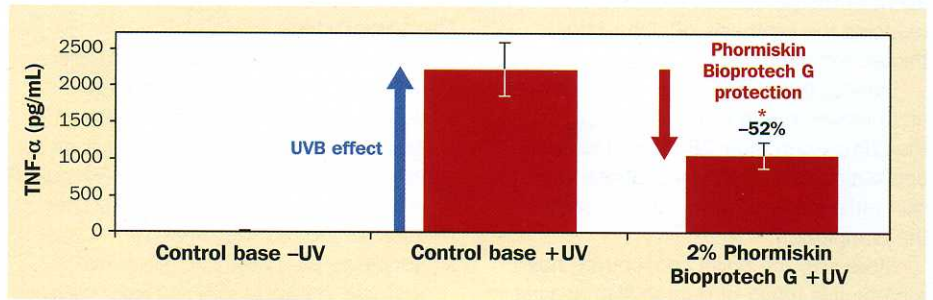
**In vivo test: rejuvenating effect**

The trial assessed the *in vivo* efficacy of a cream containing 2% of the novel concentrate on the homogeneity of the complexions of 15 healthy females aged between 45 and 65 years, having mature Caucasian skin and an uneven complexion.

The product was applied to the entire face, twice daily (morning and evening), for 28 days. The rejuvenating effect of the novel concentrate was assessed on photographs taken in polarised light.



**Figure 6:** Detection and quantification of Sunburn cells of irradiated human skin explants – treated, or not treated, with Phormiskin Bioprotech G.



**Figure 7:** Quantification of TNF- $\alpha$  production induced by UV exposure in human skin explants, after topical application of a base cream, or a cream, containing 2% Phormiskin Bioprotech G.

Two methods of analysis were used (RGB and co-occurrence matrix) to assess the efficacy of the active ingredient.

A significant reduction was observed in the colour variance of  $-2.7\%$  on average ( $p < 0.05$ ) and up to  $-11.2\%$  and a significant increase in the homogeneity of texture of  $+2.1\%$  on average ( $p < 0.05$ ) and up to  $+7.5\%$ . The lower the variance is, the greater the differences in skin colour are reduced, thus the skin colour was more homogeneous in terms of intensity of colour. The complexion is visibly more radiant and the texture of the skin is visibly finer for a “fresh complexion” effect.

An overall but not significant reduction

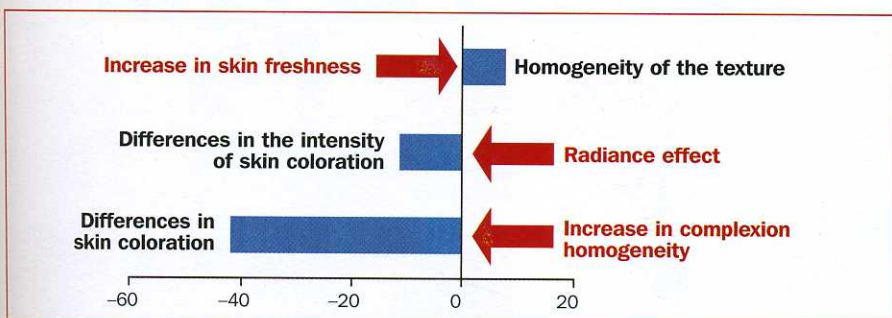
was also observed in the difference in coloration of  $-1.1\%$  on average and up to  $-41.7\%$ , corresponding to an improvement in the homogeneity of the skin (Fig. 8 and Fig. 9). The complexion is visibly more even. Moreover, the product received a good reception from the panel (Fig. 9).

**Discussion and conclusion**

Phormiskin Bioprotech G is a concentrate of original life obtained by green biotechnology from the blue micro-alga *Phormidium persicinum*. It is the result of 14 years of study, of observing nature, being inspired by it and revealing clinical properties.

The novel concentrate protects human skin cells from the ageing caused by free radicals, through the stimulation of natural defences of skin cells: the novel concentrate increases the expression of thioredoxin 1 and thioredoxin 2.

Based on these results, treatment of keratinocytes and fibroblasts by the novel concentrate increases the level of cellular thioredoxin, and this increase prior to exposure to UVA protects cells against subsequent exposure to UVA. We have previously described that when less



**Figure 8:** Optimal variations observed on 15 volunteers after 28 days of daily application of a cream containing 2% Phormiskin Bioprotech G.

affected by UV, cells produce less thioredoxin back.<sup>9</sup>

The thioredoxin system is known to stabilise vitamin C<sup>10</sup> because of its strong antioxidant power, it restores the oxidised vitamin C in its reduced form. Vitamin C (ascorbic acid) is an antioxidant and a lightener but also stimulates the maturation of collagen (it allows the maturation and export of the collagen outside the cell), thus contributing to a firming effect.

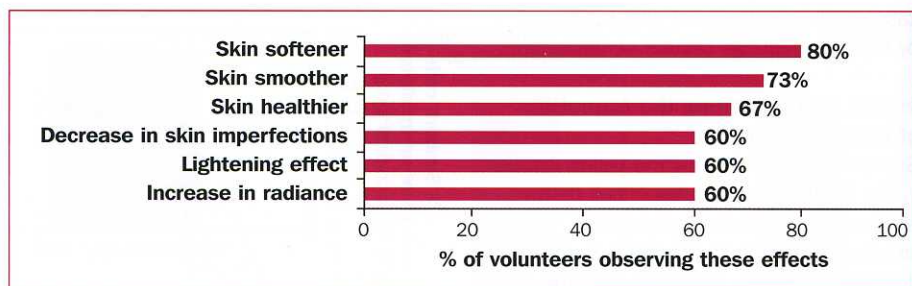
An active stimulation of the synthesis of thioredoxin would then allow recycling vitamin C and would thus, indirectly, have a firming and lightening effect; it would thus improve the homogeneity of the complexion and make skin smoother.

Furthermore, it was previously described that thioredoxin is an inhibitor of the production and secretion of melanocortin induced by UV<sup>11</sup> and melanocortin, also known as alpha-MSH, is a stimulating agent of the production melanin. By reducing the synthesis of alpha-MSH, thioredoxin improves the lightening effect.

Indeed, the *in vivo* study demonstrates an improvement in the homogeneity of the complexion after 28 days of daily application: the novel concentrate improves skin radiance and clarity, and smoothes the complexion.

Moreover, the novel concentrate has a protective effect of human skin against UV radiation. It prevents the appearance of sunburn cells (SBC). From 2%, it decreases the activation of caspase-3 in a dose-dependent manner and protects against the appearance of pyrimidine dimers. It also decreases the production of the TNF- $\alpha$  cytokine.

By fighting the mechanisms of skin ageing, Phormiskin Bioprotech G improves the homogeneity and luminosity of the skin



**Figure 9:** Evaluation, by the volunteers, of the efficacy of a cream containing 2% Phormiskin Bioprotech G.

complexion, as well as the texture of the skin. This revitalising concentrate combines reparative and protective actions. It is the ideal solution for mature skin for a rejuvenating effect combined with slowing of cutaneous ageing. This active ingredient is interesting for anti-ageing, with a novel mode of action, complementary to other well known antioxidants.

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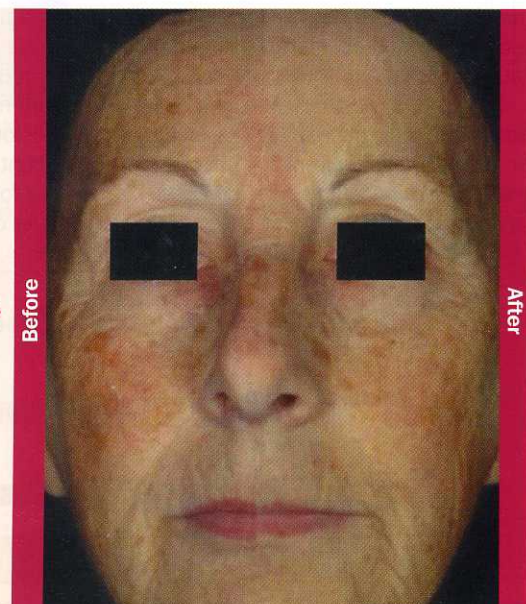
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Before



After



Before

After

**Figure 10:** The difference in skin colour before and after application of a cream containing Phormiskin Bioprotech G.