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Sustainable marine actives from biotechnology

There is an enormous potential for obtaining new active substances from the oceans. More than 230,000 known marine plant and animal species provide us each year with approximately 100 million tonnes of raw material, mainly used by the food, pharmaceutical and cosmetic industries. Sustaining and protecting marine biodiversity therefore involves improving the awareness of all the industrial and economic players.

Codif Recherche et Nature is committed to developing leading-edge biotechnological tools in order to ensure regular, equitable and high-quality provisioning of marine plant raw materials. The company therefore continues to benefit from the remarkable and innovating cosmetic properties of the marine flora, without depleting natural resources.

"Biological diversity means the variability among living organisms from all sources including, inter alia, *terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems."*

Article 2 of the Convention on biological diversity, 1992

According to an Ipsos survey, only 12% of the Top 100 cosmetic brands take biodiversity into account in their sourcing.[1] However, at least a third of the technologies used by these companies depend directly or indirectly on the living world.[2]

In 2010, biodiversity year, it has to be admitted that much still has to be done to protect biodiversity. In this context, a rapid expansion of biotechnologies seems to be the economically equitable solution between man and nature.

Aware of these problems, Codif developed an algal culture laboratory. This laboratory thereby draws the minimum natural resources required to seed cultures in the bioreactor and avoids any excessive harvesting as this would not be perennial for a species or for the ecosystem harbouring it overall.

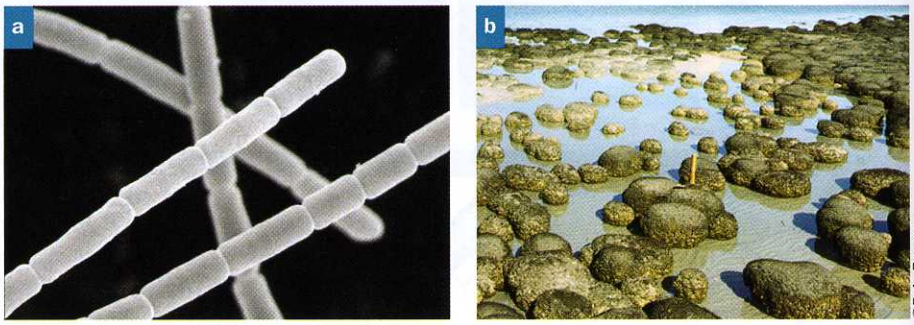

Figure 1: *Electron microscope examination of (**a**)* Phormidium persicium *and (**b**) stromatolites built by* Phormidium *visible at Shark Bay in Australia.*

There are three different types of culture: culture of microalgae and macroalgae in a photobioreactor and culture of macroalgae in the open sea illustrate our desire to develop cosmetics through the sustained and equitable use of marine biodiversity.

Growth of a blue-green microalga in a photobioreactor for the development of an anti-ageing ingredient

Phormiskin Bioprotech G is a skin rejuvenator which owes its anti-ageing properties to an immortal cyanobacterium called *Phormidium persicinum*. This microorganism belongs to the Cyanophyceae family, often referred to as the blue-green algae (Fig. 1a).

Cyanophyceae are qualified as primitive micro-organisms as they first appeared approximately 3.8 billion years ago, and form part of the species at the origin of the expansion of life on earth. Like algae, they produce oxygen by photosynthesis and this led to the filling of the earth's atmosphere with oxygen and allowed the development of life on Earth. They are also responsible for the appearance of the protective ozone layer, and the first large carbon well which decreases the greenhouse effect as the sun's temperature increases.

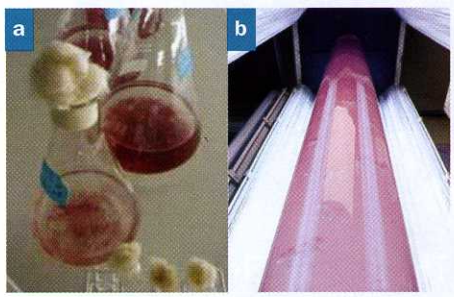

Figure 2: *Culture of* Phormidium persicinum *in (**a**) flasks, and (**b**) a photobioreactor .*

By organising itself into mucilage-producing colonies, *Phormidium persicinum* generates geological formations called stromatolites, (from the Greek strôma, carpet and lithos, stone), cushion- or pillar-shaped rocky domes (Fig. 1b). This stromatolite organisation plays several fundamental roles in the survival of phormidium in very varied ecosystems. This specific protective system has a protective effect against UV light, but also heavy metal chelation and depollution capacities. Hence, *Phormidium* lives nearly everywhere, including under extreme conditions, from polar ice to desert sands. It survives in the very hot and/or acid lakes of volcanic craters and in geysers.

These remarkable adaptive capacities are characteristic of highly effective enzymatic systems which may therefore be used for cosmetic applications. However we very rapidly came up against the problem of provisioning. As it is impossible to collect *Phormidium persicinum* in its natural environment, Codif Recherche et Nature research laboratories developed cultures of the microalgae in flasks and then in photobioreactors (Fig. 2). Three years of

work were necessary to define the best culture medium and growth conditions (illumination, oxygenation, pH etc.) which are similar to those of the original environment of the microalgae. These cultures have been scaled up to industrial level and now provide the necessary quantities for the marketing of the active cosmetic ingredient Phormiskin Bioprotech G, without again ever drawing resources from the marine environment.

Culture of a macro-alga in the open sea for the development of a replumping agent

Dictyopteris membranacea, is a brown alga of the European Atlantic coasts belonging to the Dictyoptales family, Dictyotaceae. Its thalli form membranous blades or thin ribbons measuring from 10 cm to 30 cm and divide by dichotomy.

This alga is found in the rocks of the lower littoral level and at depths of up to 80 m so that it may only be harvested by diving. *Dictyopteris membranacea* is a protected species in the Mediterranean. The concept of protection of biodiversity is therefore essential.

In this context, Codif has developed an original algal culture programme on a 70 hectare marine leasehold site in a protected zone at the mouth of the Rance river, thereby ensuring regular provisioning without depleting natural resources.

Several culture tests were performed, and finally the capture method was selected which is used in particular to culture mussels. This method has the advantage of being natural without requiring chemical treatments.

To seed the first cultures, *Dictyopteris* algae are harvested from the wild during the summer, then cleaned several times with sterile sea water. Sterilisation is mechanical and is performed by ultrafiltration (0.2 µM).

The hatchery phase then begins: these algae issue spores which bind to small ropes laid out in growing racks called collectors (Fig. 3a).

Dictyopteris has a direct biological cycle of the monogenetic type. After approximately three weeks, the spores firmly adhere to the rope and germinate and develop until they become small algae identical to their parents. The culture phase then follows this hatchery phase: the small algae are transferred to the open sea in culture zones on rope lines (Fig. 3b). They are harvested one year later in the autumn. The spores derived from these cultured plants are then replaced in the hatchery in order to initiate a new multiplication cycle.

The aromatic fragrance of *Dictyopteris membranacea* is very characteristic and gives certain bays (Hawaii) a marine odour due to the presence of dictyopterene-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.

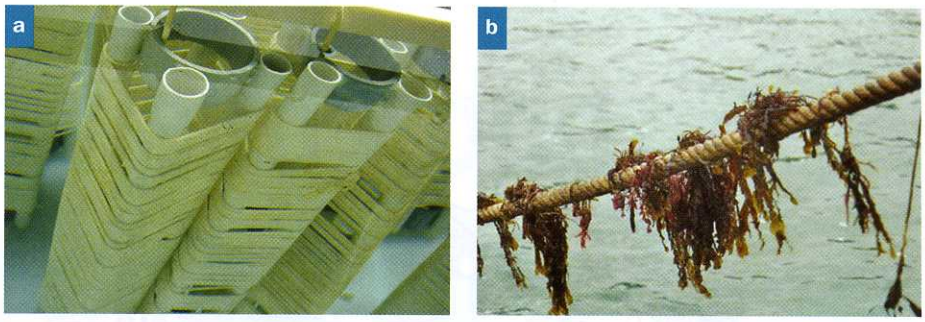

Figure 3: *(**a**) Culture of* Dictyopteris membranacea *spores in the hatchery, then (**b**) young algae in the sea.*

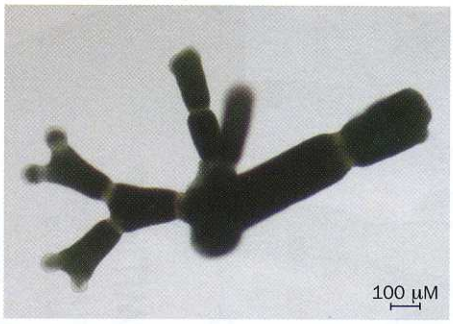

Figure 4: *Axenic microthallus of* Jania rubens.

Photobioreactor culture of a calcareous macroalga used for its anti-ageing properties

Jania rubens belongs to the Corallinaceae family of calcareous macroalgae often described as marine vegetable coral. Their tree-like structure holds many living organisms such as microalgae, micro-organisms or small invertebrates, and itself forms an ecosystem promoting marine biodiversity.

As these algae have a very slow growth rate, uncontrolled harvesting associated with poor management of this resource could be fatal for perpetuation of the species, and that of all the other species which it lodges. To safeguard this biodiversity as well as guarantee the provisioning of a high-quality biomass,

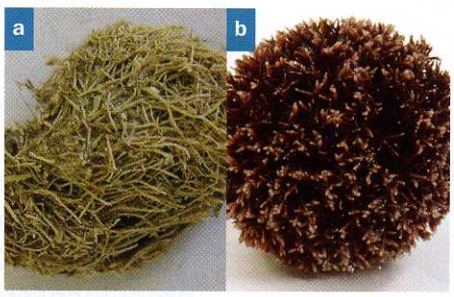

Figure 5: *Thallus of (**a**) wild, and (**b**) cultivated* Jania rubens.

Codif laboratories have developed a closed photobioreactor culture procedure. Culture of calcareous macroalgae has never been described and/or achieved before.

The association of physical and chemical treatments which have no impact on the nature of the alga, has made it possible to isolate axenic microthalli of *Jania rubens* from 1 mm long fragments harvested in Brittany and Corsica (Fig. 4).

The optimisation of culture conditions (medium composition, illumination and agitation), and scaling up to a closed 300-L photobioreactor run in fed-batch mode makes it possible to obtain a biomass of higher quality than the wild species (Fig. 5), with twice the growth rate. Under optimum culture conditions, the calcium and magnesium concentrations of the biomass are the same as for the natural biomass: 23.6% and 2.6% dry weight respectively.

Because of its high bioavailable calcium content, *Jania rubens* is a promising candidate for the development of an anti-ageing, moisturising and remineralising cosmetic ingredient.

Conclusion

Despite the time and work required for its implementation, culture of micro or macroalgae in a bioreactor or the open sea has many advantages. The first advantage is the sustainable and perennial management of marine natural resources and their biodiversity together with the permanent availability of a resource independent of environmental factors. A further advantage is that the biomass obtained has an identical or even higher quality than that obtained in the wild and retains its property of producing active molecules or substances with useful and always innovating cosmetic applications. PC

References

1 *Expression Cosmetique*. 2010 May-June 03, 107.
2 Weber J. Accounting for Changes in Biodiversity and Ecosystem Services from a Business Perspectives. Cosm'ing 2010.