

# Marine holobionts: Novel multi-functionals

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Holism is a philosophical notion first proposed by Aristotle in the 4th century BCE. It states that systems should be studied in their entirety, with a focus on the interconnections between their various components rather than on the individual parts. Such systems have emergent properties that result from the behaviour of a system that is greater than the sum of its parts.

The key to life is cooperation. From the most primitive photosynthetic life forms through plant and animal organisms, each individual (or macrobiont) is the host that harbours and cooperates with its microbiota. As a whole, each organism is a unique biological functional entity, a system, and must be studied as such.

Almost half a century ago, Lin Margulis coined the concept of a 'holobiont', a superorganism composed of a macrobiont and its own microbiota. The concept is a renewed paradigm in biology that can help to describe and understand these complex systems.

Although initially driven by studies of marine organisms, much of the research on the emerging properties and significance of holobionts has since been carried out in other fields of research: the microbiota of the rhizosphere of plants or the animal gut became predominant models and have led to an ongoing paradigm shift in agronomy and medical sciences.<sup>1</sup>

We find all kinds of holobionts in nature. Each cell of marine phytoplankton is surrounded by a microenvironment called the phycosphere, where it interacts closely with its microbiota; on land, plants create the rhizosphere in their roots, where they harbour their microbiota; and human beings live with their microbiota on the skin, in an intimate environment called the dermosphere. (Figure 1).

Phytoplankton, plants and human holobionts have surprising things in common. Firstly, they

all develop an intimate microenvironment of interaction, where a fascinating metabolic exchange takes place: the phycosphere, the rhizosphere and the dermosphere, respectively.

The macrobiont chemically alters the microenvironment by modifying oxygen and pH levels, releasing nutritive organic compounds and producing chemoattractants, such as amino acids, carbohydrates, sugar alcohols and organic acids, to favour the colonisation of the microbiota. The microbiota, for its part, provides a series of metabolites that help train the immune system of the macrobiont, as well as supplying vitamins and metabolising organic compounds.

The most striking and fascinating thing is that, whatever the holobiont, nature has chosen a microbiota composed of very similar microbial families. The ocean and the land have a homology of about 70% with our own microbiome. For example, *Lactobacillus* bacteria and ammonia-oxidising and -nitrifying bacteria both help restore a healthy skin microbiome. These bacteria have populated not only our skin microbiome naturally, but also that of the land and ocean organisms.<sup>2</sup>

## Marine holobionts tales

Host-microbe interactions play a crucial role in marine ecosystems. Sponges and corals sustain entire ecosystems through their participation in nutrient cycling by their microbial partners. Corals provide ammonia compounds and zooxanthellae microalgae provide carbohydrates to corals. The sponges metabolise organic compounds for their microbiota and the microbiota provide vitamins. As entities, these holobionts help to regulate the flux of nutrients in coral reef ecosystems.

Phytoplankton have 2.7 billion years of metabolic experience. More than 326 species

## ABSTRACT

Holism is a philosophical notion that states that systems should be studied in their entirety, with a focus on the interconnections between their various components rather than on the individual parts. The holobiont concept unifies the host-microbiota interaction and invites us to seek holistic solutions for skin health and skin microbiota. In this article we dive into the origin of this concept and the connection between the marine and human holobionts. We also present for the first time a new multifunctional cosmetic ingredient based on the molecular richness of holobionts and the ubiquity of molecules that nature has left us as a gift to combine health and beauty.

of phytoplankton depend on the B-complex vitamins provided by their microbiota for their growth. In return, their microbiota receives carbohydrates. In some cases, the bacteria also act as a defensive shield against reactive oxygen species.

At the systemic level, a state of equilibrium (eubiosis) in the phytoplankton holobiont is essential for their own survival and for life on Earth: they are the first link in the food chain, regulate the nutrient cycle in the oceans and play a role in balancing the climate<sup>3</sup> (Figure 2).

## The problem

Under ideal conditions, our skin can maintain eubiosis and be protected against the impact of external factors, such as UV radiation (UVR), pollutants and pathogen agents, and internal physiological states resulting from, among other causes, chronic conditions, oxidative stress or genetic predisposition.

Almost 54% of the world's population live in cities, where we are subjected to urban pollution, overexposure to UVR, stress and industrial food. In these circumstances, our own health and that of our microbiota can be affected.

Extensive scientific evidence reveals that an imbalance in the composition of our skin microbiota (dysbiosis) affects skin barrier function. It is intimately related to premature ageing and all kinds of skin diseases and conditions, including atopic dermatitis, acne and psoriasis.

Dysbiosis states can be reverted to a eubiosis state by maintaining a healthier lifestyle and

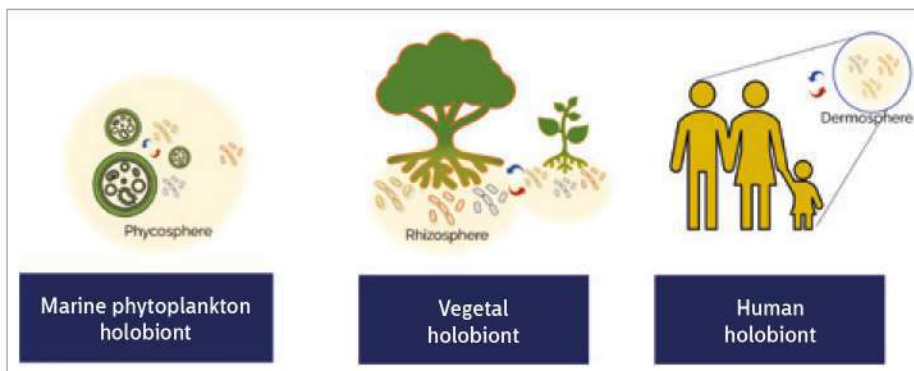


Figure 1: Holobiont conceptual model: From marine to human holobiont

establishing daily skin care routines. In this sense, the use of natural products with multi-functional effects is essential to protect the skin tissue and our microbiota.

### A novel solution

Holobiont extracts are unique natural source - they are the essence of an entire ecosystem. The secondary metabolites produced by the phytoplankton-microbiota interaction are released to the phycosphere.

These molecules, in addition to having a nutritional function, have shared functional effects as antioxidants, photoprotectors, regenerants, moisturisers, or antibiotics against pathogenic organisms to maintain the integrity of the holobiont. Fortunately, many of them are present in all living things and can therefore be functional if they are part of a bioactive ingredient.

This constitutes a paradigm shift from traditional monospecific microbial extracts to multi-species microbial extracts. Holobionts offer the molecular richness of each species and the extracellular molecules resulting from the interaction between the host and its microbiota (Figure 3).

By respecting the integrity of holobiont extracts, it is possible to preserve this molecular richness to obtain multiple biotic effects in multi-functional dermo-cosmetic products: nourishing and protective substances for skin tissue, molecules with a prebiotic effect, to re-establish the balance of the microbiota (eubiosis) and molecules with a postbiotic effect. Together, these molecules can exert a synergistic beneficial effect with a systemic impact on the health and healthy appearance of the skin.

In this new era of skin health and care, there is a growing awareness, especially among young consumers, about the use of new, value-added products with a focus on skin microbiome and, of course, microbiome-friendly. The impact of this new claim, which is constantly evolving, opens up a great opportunity for cosmetic brands that are committed to new ingredients to find a blue ocean in the market.

### Blue tech to produce marine holobiont

Based on its knowledge of phytoplankton-microbiota interactions, European biotechnology company Microalgae Solutions

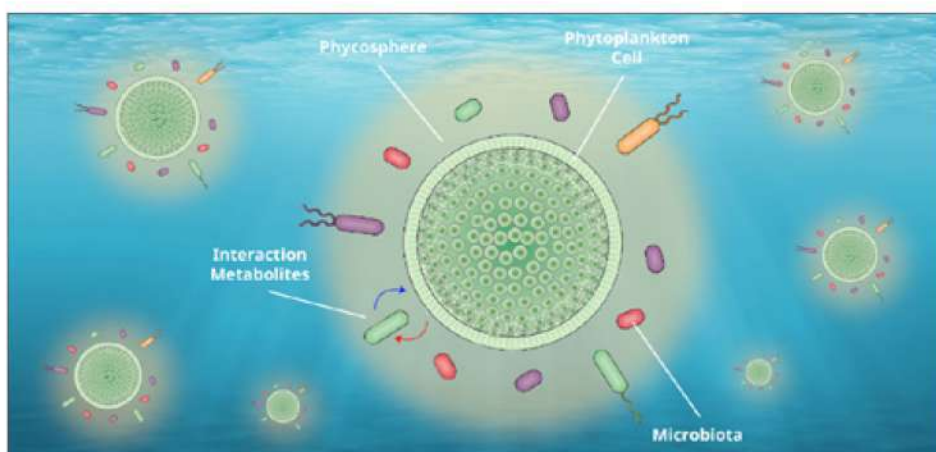


Figure 2: Phytoplankton holobiont

(MAS) has discovered the functional mechanisms of marine holobionts to obtain extracts of unique molecular richness.

Inspired by nature and using its own technology, Phycosphere Biodynamic® Technology (PBT), MAS obtains phytoplankton holobiont extracts. PBT is a patented, environmentally friendly process that uses less energy and water than other microalgae production systems. The technology combines bioengineering and green lab-grown techniques to biomimic the marine micro-ecosystem where holobionts live.

By providing the right lighting conditions in terms of wavelength and photoperiod, an adequate level of nutrients (nitrogen, phosphorus, potassium and micronutrients) and a source of gas exchange (CO<sub>2</sub>), the phytoplankton holobionts reach an optimal growth and equilibrium state. This way we obtain maximum performance, enhancing the production of the metabolites involved in maintaining the state of eubiosis called cell redox homeostasis.

A proteomic data analysis of the marine phytoplankton holobiont (Figure 4) reveals that, in the exponential growth phase, close to 60% of gene expression is induced to encode metabolites involved in cell redox homeostasis and about 30% encoding for metabolites involved in carbohydrate metabolism, the major extracellular matrix component of the phycosphere.

Bioactive molecules with diverse cosmetic claims can be identified in the same extract, giving them multi-functional properties (Table 1). Complementary biochemical studies have also identified other antioxidant molecules of dermo-cosmetic interest: phytopigments, polyphenols and organic acids.

PBT is part of the 'blue bioeconomy'. This technological sector is recognised by the EU as a priority environment to promote the development of new products of marine origin, as an alternative to traditional industrial products and services, which generally have a high environmental impact and are part of the linear economy<sup>4</sup>.

To date, 22,000 metabolites have been obtained from the vast marine wealth of the world. Of these, 60% come from microorganisms and their applications cover biomedicine, biomaterials, nutraceuticals (functional foods), agricultural products and bioremediation.

In cosmetics, there is a great opportunity for ingredients from blue bioeconomy companies, due to their multifunctional properties and their marine origin. Marine compounds can be incorporated into skincare and make-up products. Their drug-like benefits lead to pharmaceutical hybrids where bioactive ingredients are added to topical or oral cosmetics to produce a cosmeceutical with enhanced properties.

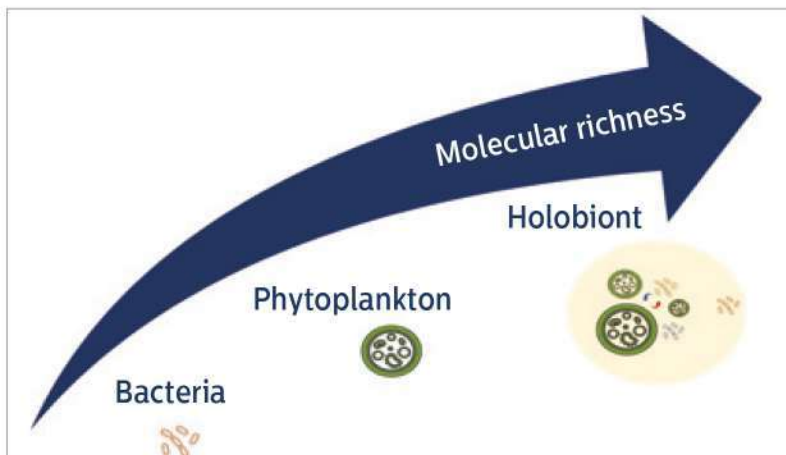


Figure 3: How holobionts develop

TABLE 1: BIOACTIVE MOLECULES WITH DIVERSE COSMETIC CLAIMS IDENTIFIED IN PHYTOPLANKTON HOLOBIONT EXTRACT

Bioactive molecule biosynthesis	Dermocosmetic properties
Glutathion peroxidase (GPx)	Antioxidants
Superoxide dismutase (SOD)	
Folic acid	
Vitamins A,C,E & B complex	
Phytopigments (B-carotene, Ficocyanin)	
Thioredoxin (TRx)	Reduce inflammation
Isoleucine & valine	well aging
p-coumaric acid	Skin-lightening
Genistein (phytoestrogen)	Probiotic (Sebum regulator)
Sucrose	Humectant moisturised
	Exfoliant
	Prebiotic
Trehalose	Solar booster (UVA & blue light)

Phycoskin® (PSK) bioactives are high value-added marine extracts biotechnologically developed by MAS and are the first cooperative ingredients from marine holobionts. They are produced under strict internal quality standards and to COSMOS-certified quality.

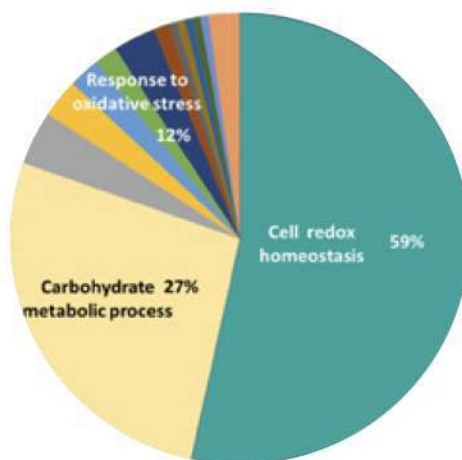
The extraction process uses unique green chemistry principles and do not involve the use of environmentally harmful chemicals. Thus, they are non-sensitising, non-dermal and ocular-irritating.

The formulation stability and high skin penetration capacity of a state of equilibrium is possible thanks to a proprietary encapsulation technology developed by MAS. Molecular Carrier Biotech (MCB) keeps the odour, oxidation and colour problems, which are very common in extracts containing phytoplankton, to a minimum.

### Antioxidant-whitening dual system

PSK One, hereafter referred to as planktonic holobiont, comes from a holobiont composed of Atlantic and Mediterranean plankton species. This marine holobiont bioactive releases the antioxidant-whitening dual system to comprehensively combat the effects of exposome on skin ageing and pigmentation.

The antioxidant action of this holobiont combines the effect of primary (the enzymes SOD and GTx) and secondary antioxidants (vitamins A, C and E, and phytopigments), providing an anti-melanogenic shield. The whitening action is based on the effect of p-coumaric acid, as a



**Figure 4:** Proteomic data analysis of phytoplankton holobiont extracts

tyrosine inhibitor that prevents and reduces the skin spots. The brightening effect is due to the melanin breakdown action of lignin peroxidase, balancing skin tone.

*In vitro* studies showed that planktonic holobiont at 0.3% has antioxidant properties that combat the signs of photoageing. An anti-glycosylation effect of +34% and a trolox capacity of +58  $\mu\text{m}$  was recorded. An enzymatic study revealed a +50% hyaluronidase inhibition effect. The bioactive also demonstrated a brightening effect, due to its +51% tyrosinase inhibition capacity.

The clinical efficacy of planktonic holobiont was tested, *in vivo* during the summer months, with a blind, placebo-controlled study. The studies were conducted with 21 volunteers, between 29 and 65, who had been exposed to exposome factors (UVA and pollutions) and most had clear signs of hyperpigmentation.

They applied the bioactive at 1% on the skin of the face, twice daily for 28 days. Baseline moisture, elasticity and pigmentation were measured on Day 0 by corneometer, cutometer and mexameter, respectively, and after 28 days using a Microcaya multi-dermoscope.

As Figure 5 shows, moisture and elasticity level were increased by 24% and 18% respectively, showing that the provides lifting and moisturising against exposome effects. It reduced pigmentation by 30% in the spot (area with hyperpigmentation), but only by 7% outside, indicating a spot reduction effect and balancing the skin tone and radiance.

### Synbiotic effect for skin balance

Synbiotik Phycoskin comes from a benthic holobiont composed of phytoplankton species from rocky shore microecosystem. This benthic holobiont bioactive provides a neobiotic molecular complex composed of postbiotic substances that restore the skin balance and prebiotic substances that rebalance microbiota, reducing visible dysbiosis impairment, such as redness and lesions in acne-prone skin.

*In vitro* studies showed that benthic holobiont bioactive at 0.05% promotes 21% growth of *Staphylococcus epidermidis* against *Staphylococcus aureus*, providing a prebiotic effect. Therefore, if the *S. epidermidis* and other beneficial bacteria may proliferate against *S. aureus* colonisation, resulting in a rebalance of skin microbiota (eubiosis) and skin tissue recovering.

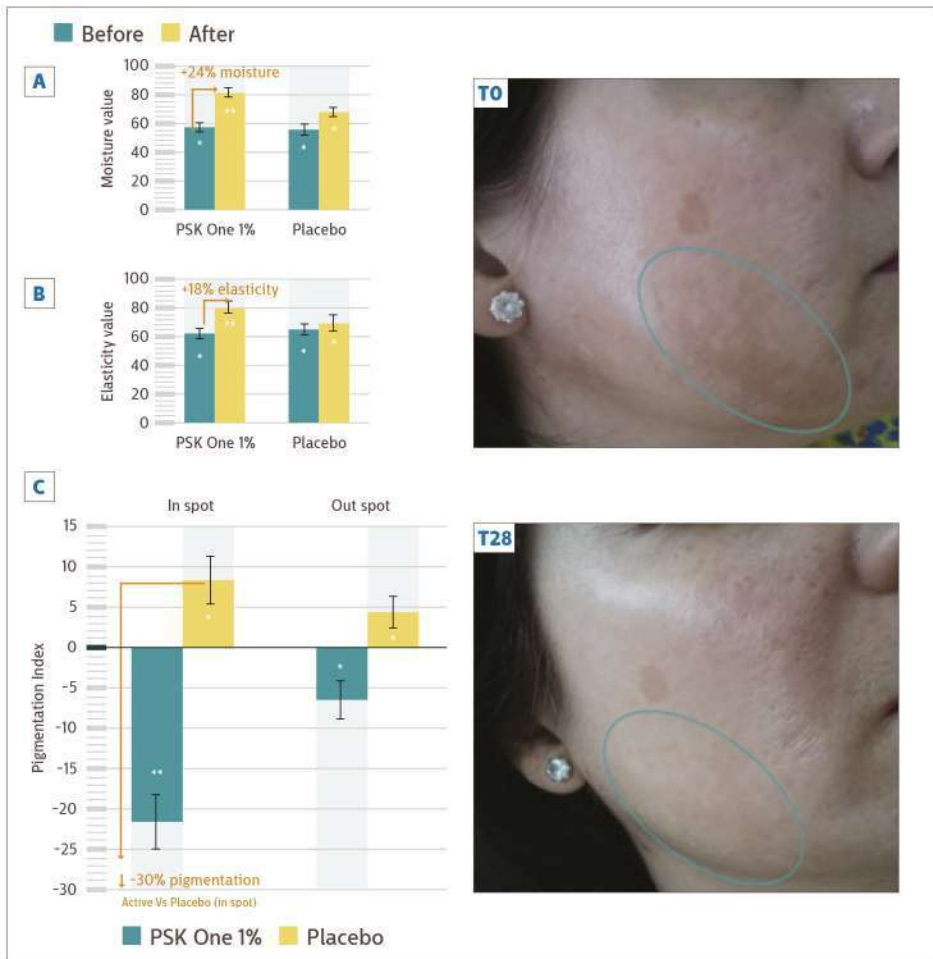
The clinical efficacy of benthic holobiont bioactive was evaluated *in vivo*, measuring the main parameters to establish the skin condition: stratum corneum moisture, sebum content and skin surface pH. The double-blind study was conducted during the COVID-19 pandemic, with 40 volunteers aged 19 to 29, who applied 1% bioactive on their faces twice daily for 28 days. They had acne-prone skin and had used facemasks before and during the study, so they were expected to be prone to 'maskne'.

As Figure 6 shows, the benthic holobiont bioactive significantly improved (68%) the volunteers' moisture index. It was more noteworthy on the T zone (forehead and chin) and the cheekbones. It also significantly reduces sebum index by 37% in all volunteers with moderate and severe acne after four weeks of use.

### Formulation examples

Due to the MCB system, these bioactives are superbly stable ingredients in final formulas. They are of >99% natural origin content (ISO 16128), microbiota-friendly and water-dispersible. The main applications are in night and daily creams and serums, dermocosmetics and eye contour creams.

The key benefits of the planktonic holobiont bioactive are:



**Figure 5:** *In vivo* study of PSK One

Note: Comparative values of moisture (A), elasticity (B) and pigmentation index (C) and pictures of a volunteer from Day 0 to 28 using PSK One at 1% twice a day

- Antioxidant action: Exposome and life-style balancing, natural skin defence boosting, anti-pollution & detox treatment.
- Whitening properties: Brightening & lightening treatment, skin tone evener, spot fading treatment

For the benthic holobiont bioactive, the key benefits are:

- Prebiotic action: Microbiota balance, natural skin defence boosting, preventing & repairing acne lesions & redness
- Postbiotic action: Lifting effect, skin shine reduction

As examples, Tables 2 and 3 show the formulation of a moisturising, lifting, brightness and even tone cream and a microbiota balance maskne repair and regulator serum.

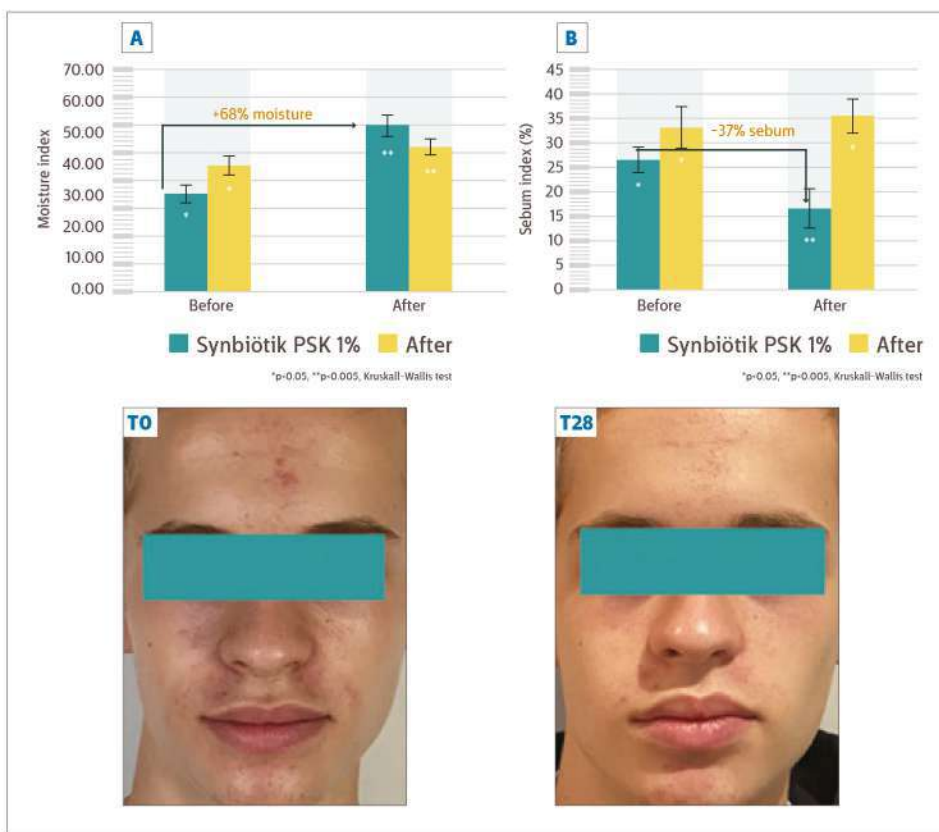
## Conclusions

The holobiont concept unifies the host-microbiota interaction and can help us to integrate the systemic point of view to find new solutions and offer new multi-functional products, for the benefit of our skin and its microbiota. Holobiont extracts are a unique natural source of molecules for the production of multifunctional ingredients with biotic effects that have a systemic impact on the health and healthy appearance of the skin.

Phycoskin® actives are a new generation of cosmetic ingredients based on high added value marine extracts. PSK One provides a moisturising lift against the effects of exposure and also reduces pigmentation levels by balancing skin tone and increasing radiance. Synbiötik PSK provides a prebiotic effect, increasing the growth of beneficial bacteria in the skin's microbiota and postbiotic effects, restoring the skin's balance: reducing sebum production and lesions in acne-prone skin. **PC**

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**Figure 6:** *In vivo* study of Synbiötik PSK

Note – Comparative values of moisture (A) & sebum elasticity (B) index & picture of a volunteer after 28 days using Synbiötik PSK at 1% twice daily

**TABLE 2: MOISTURISING, LIFTING, BRIGHTNESS & EVEN TONE CREAM WITH PHYCÖSKIN ONE**

Phase	Ingredients	INCI	% w/w
A	Water	Aqua	q.s. 100
	Glycerin	Glycerin	10
	Agar-agar	Agar	1.5
	Pentano-1,2-diol	Pentylene glycol	0.85
	3-(2-ethylhexyloxy) propane-1,2-diol	Ethylhexylglycerin	0.15
B	Sunflower seed oil	Helianthus annuus seed oil	18
	Gliceril stearato	Glyceryl stearate	5
	Vitamin E	Tocopherol	0.3
C	PSK One	Caprylyl capryl glucoside, water, helianthus annuus, seed oil, plankton extract, potassium sorbate, sodium benzoate, tocopherol	1
D	Fragrance	Parfum	0.5

**Procedure:** Stir Phase A at 75°C until it forms a homogeneous mixture. Stir Phase B at 75°C until it forms a homogeneous mixture. Disperse Phase B into Phase A and emulsify using homogeniser until a homogeneous mixture for ten minutes. Cool to room temperature, stirring constantly. Add Phase C at room temperature. Add Phase D to phase A, B and C, and emulsify until for five minutes with 3,400 rpm until it forms a homogeneous mixture.

**TABLE 3: MOISTURISING BALANCE MASKNE REPAIR & SEBUM REGULATOR SERUM WITH SYNBİÖTİK PHYCÖSKIN**

Phase	Ingredients	INCI	% w/w
A	Water	Aqua	q.s. 100
	Hydrolysed sodium hyaluronate	Hydrolysed sodium hyaluronate	1.30
B	Potassium sorbate	Potassium sorbate	0.5
	Sodium benzoate	Sodium benzoate	0.5
C	Synbiötik PSK	Caprylyl/capryl glucoside, water, helianthus annuus seed oil, pentylene glycol, plankton extract, ethylhexylglycerin, tocopherol	1
D	Fragrance	Parfum	0.3

**Procedure:** Homogenise Phase A. Add Phases B, C and D, keep stirring.