DEINOVE

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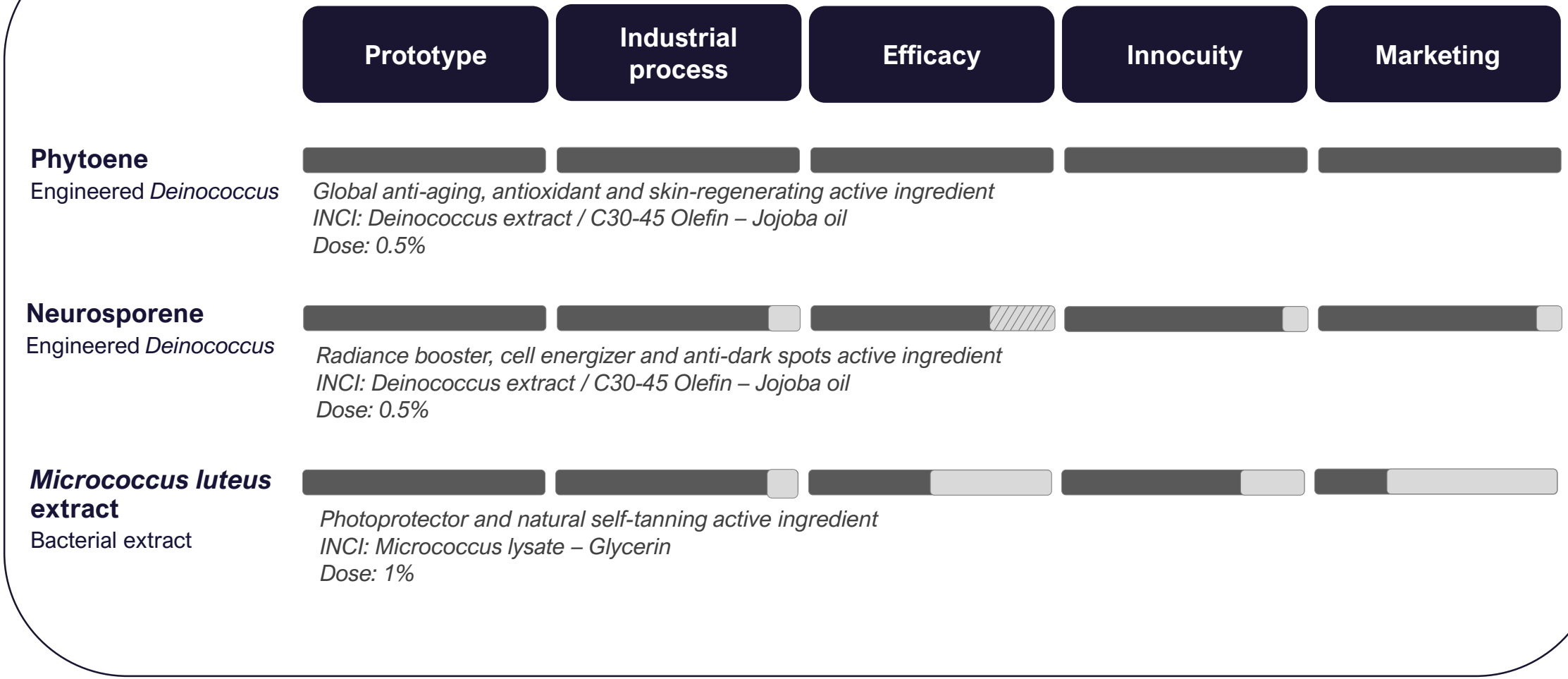
DEINOVE's active ingredients portfolio



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Phytoene

Phytoene

Global anti-aging, antioxidant and skin-regenerating active ingredient

- **Properties:**

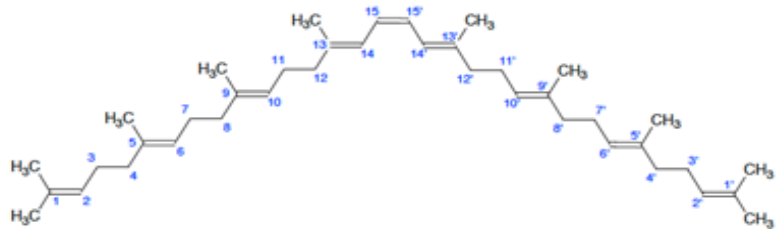
✓ The precursor of all carotenoids

✓ A colorless molecule

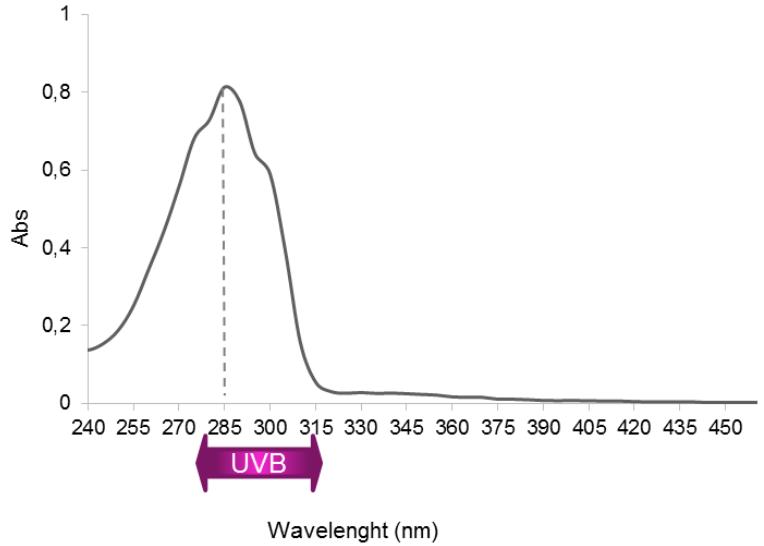
✓ The only carotenoid absorbing UVB

✓ Lipophilic molecule

✓ Need a sustainable sourcing



- Dietary phytoene intake is coming from vegetables and fruits1.
- Daily intake within an equilibrated regimen is estimated around 2 mg/day2.
- Phytoene accumulates within the skin at a level around 65 ng/g of skin1.



1. Khachik et al. (2002). Experimental Biology and Medicine (Maywood, N.J.), 227(10), 845–51.
2. Biehler et al. (2012). Journal of Food Composition and Analysis, 25(1), 56–65.

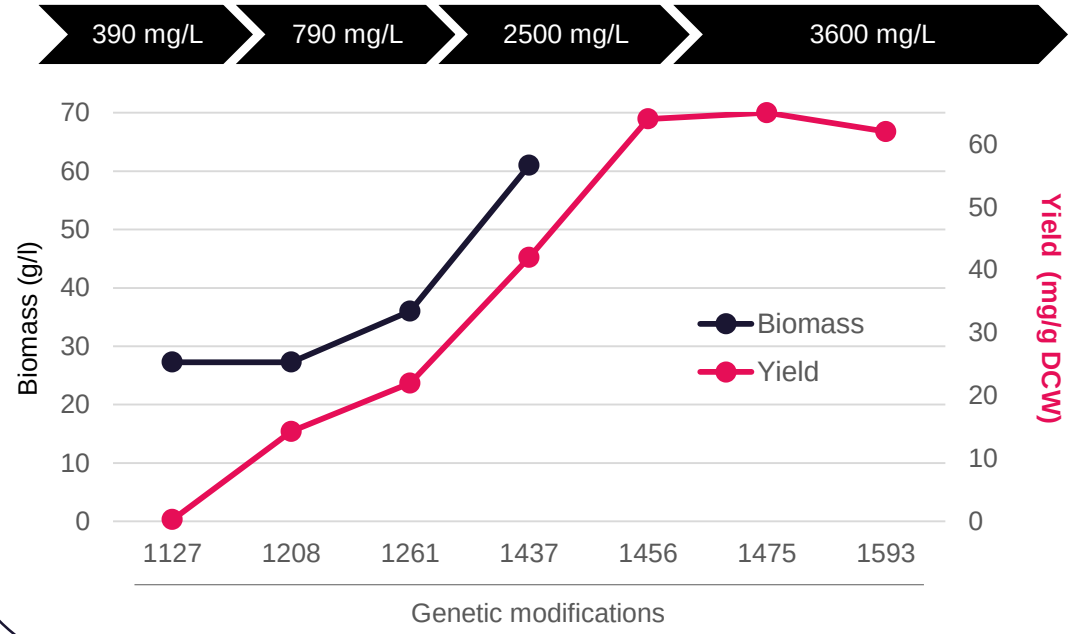
Phytoene

Development of an industrial compatible process

Prototype

1. Strain development and fermentation process

Combined progress in genetic engineering and fermentation to improve performances: **x540**



2. Extraction, purification, formulation

- Process scalable up to industrial scale
- Use of solvent compatible with cosmetic rules
- Absence of heavy metals, CMRs, allergens…
- Stable ingredient

➢ Process development to secure upscaling

➢ Process advantages:

✓ Independent of seasonality and climate
✓ High concentration of molecules
✓ Sustainable raw materials
✓ No preservatives

Phytoene
Scale-up success

Industrial process

DEINOVE development → **CDMO transfer and production**

Fermentation:

- ✓ Process development of this hardly cultivable thermophilic strain
  - ❖ Mineral medium
  - ❖ Fed batch process of 96h
  - ❖ Exponential feed rate

Downstream:

- ✓ Process development
  - ❖ Diafiltration
  - ❖ S/L separation (centrifugation)
  - ❖ Maceration
  - ❖ S/L separation (filtration)
  - ❖ Evaporation
  - ❖ Formulation

Industrial transfer: 4 batch were produced in 2m³ scale

- ✓ Process book validated
- ✓ Process robustness
- ✓ Process repeatability
- ✓ Lab scale performances recovered

Phytoene

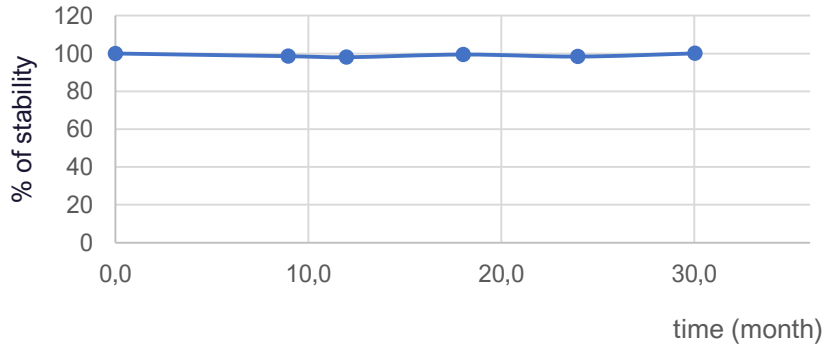
Stability confirmed over 30 months at room temperature

Industrial process

- **Stability study**

Organoleptic evaluation and analytical control (HPLC) for 2 months at 45°C & 50°C and 18 months at 4°C & 20°C

Stability evolution of PHT batch 20044V4 over 30 months at 20°C



➢ Phytoene, a colorless to yellow solution, remains stable over 30 months at 20°C

- **Stability study in a cream**

Organoleptic evaluation and analytical control (HPLC) for 1 month at 50°C and 3 months at 4°C, 20°C & 42°C

T0	3 months at 20°C
White cream Iris cotton odor *Phytoene*: 44.5 ±1.7 (mg/kg)	White cream Iris cotton odor *Phytoene*: 48.4 ±0.2 (mg/kg)

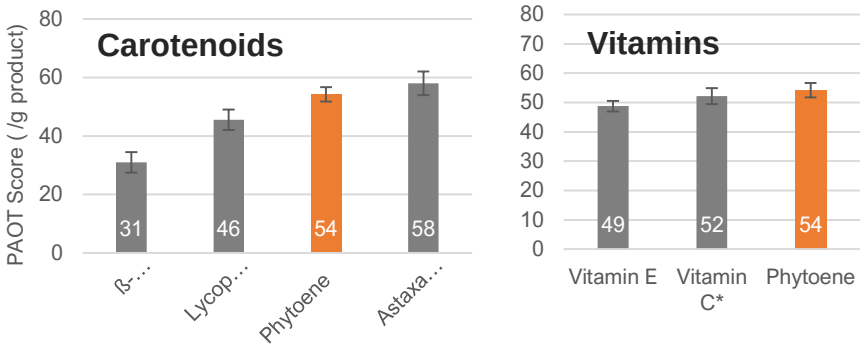
➢ Phytoene remains stable in a cream

<u>NB:</u> A complementary study shows that phytoene is also stable in olive oil, squalane and isoamyl laurate. Phytoene is also stable in jojoba oil from 2 different suppliers.

Phytoene
A potent antioxidant

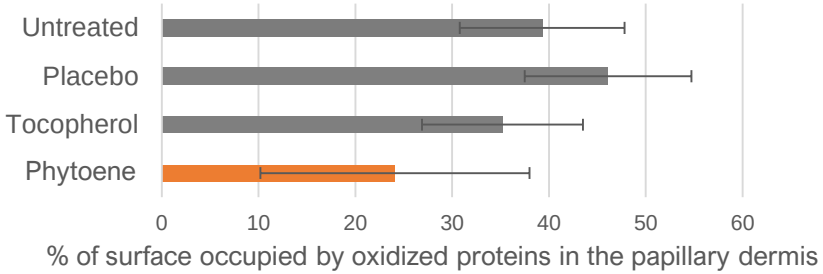
Efficacy

***In vitro* PAOT test**



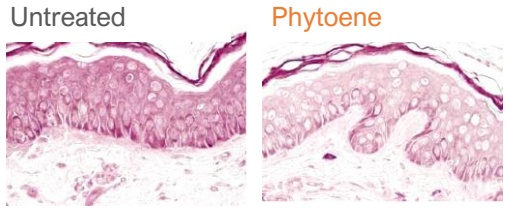
➢ **Powerful antioxidant** compared to carotenoid & vitamin benchmarks

***Ex vivo* protein oxidation**



➢ **Protection of proteins** from oxidation by phytoene: -40%

***Ex vivo* lipid peroxidation products** under UV irradiations

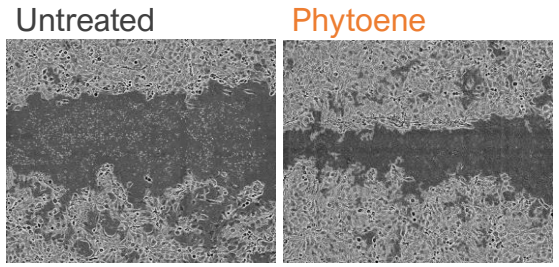


➢ **Protection of lipids** from oxidation caused by UV irradiations by phytoene: -79%

Phytoene
A potent antioxidant

Efficacy

***In vitro* scratch test**
Cell repair
23h after a scratch



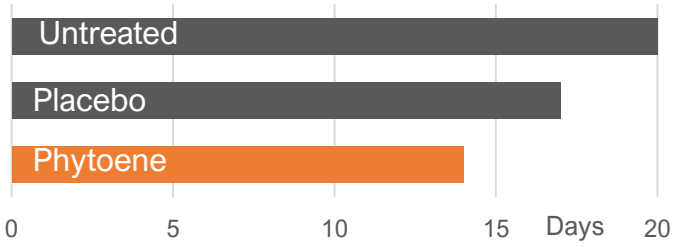
➢ **Skin repair acceleration** by Phytoene (x1,5)

***In vitro* proteomic analysis**

52% of proteins significantly impacted by phytoene (x1,5) are involved in skin regeneration

➢ **Upregulation of Laminin-5** by phytoene (x1,9)

***In vivo* skin regeneration**
Days before total skin regeneration (DHA test)



➢ **Complete skin regeneration** by phytoene after 14 days

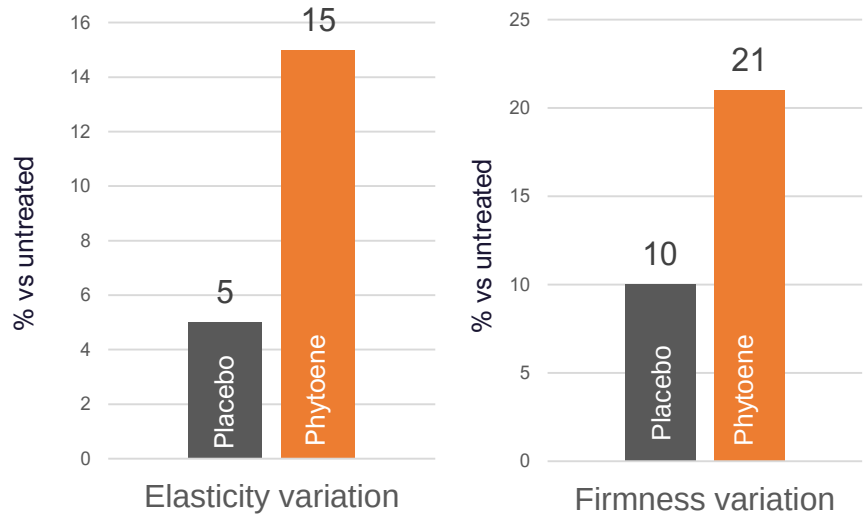
Phytoene

Wrinkles reduction tested clinically

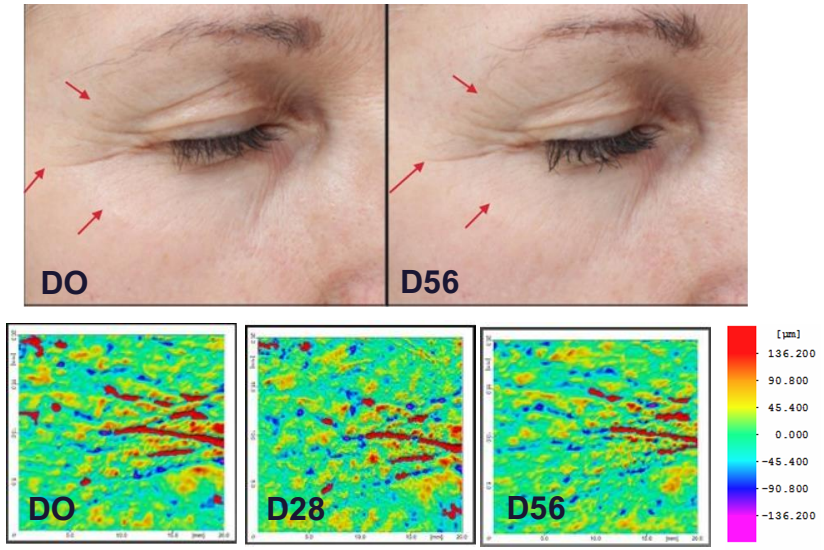
Efficacy

Clinical study: 15 women, ± 55years old, half face application, twice/day for 56 days

Cutometer test



VISIA test & AEVA test



- **Significant improvement of skin elasticity** (x3) and **skin firmness** (x2) by phytoene after 56 days.

- **Improvement of skin texture and wrinkles reduction** by phytoene: -17% after 56 days. No improvement with placebo.

Phytoene

Global anti-aging, antioxidant and skin-regenerating active ingredient

Prototype	• Sustainable sourcing: extremophile microorganism collected from a water source in Pyrenees • Strain optimization to get a stable high yield phytoene producing strain in reactor • Fermentation of natural sugars by *Deinococcus*, extraction, purification and formulation in jojoba oil
Industrial process	• Robust and reproducible controlled process, good extraction yield, fast process, good traceability • Upscaling successful (4 industrial batches to date) • Stability confirmed: 30 months at room temperature
Efficacy	• Highly efficient at low dosage (0.5%) for skin protection and repair thus promoting a youthful skin • Identified mode of action to enhance skin regeneration based on Laminin-5 • Clinically tested for its anti-aging properties: anti-wrinkle, skin firming, skin elasticity
Innocuity	• Safety confirmed with 100% of the active ingredient (1% phytoene): skin irritation (SkinEthic), skin sensitization (KeratinoSens / in silico / Direct Peptide Reactivity Assay), ocular irritation (Het-Cam), skin compatibility (Patch test), phototoxicity, reverse mutation assay in bacteria (Ames test)
Marketing	• Original storytelling: *Deinococcus,* an extraordinary microorganism famous for its extreme resistance • 100% Made in France, 100% natural origin (ISO 16128), easy to use in various cosmetic products

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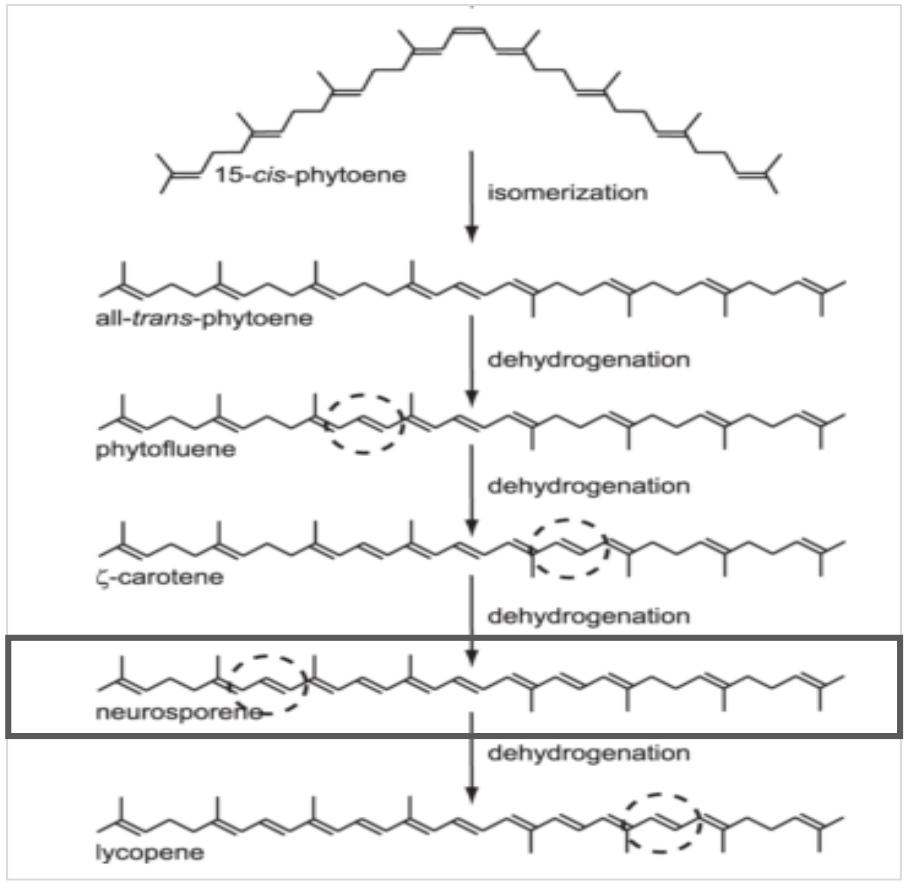
Neurosporene

Neurosporene

DEINOVE's expertise to obtain a stable strain in fermenter producing a targeted molecule



One step reaction

Phytoene

18 months

13 months

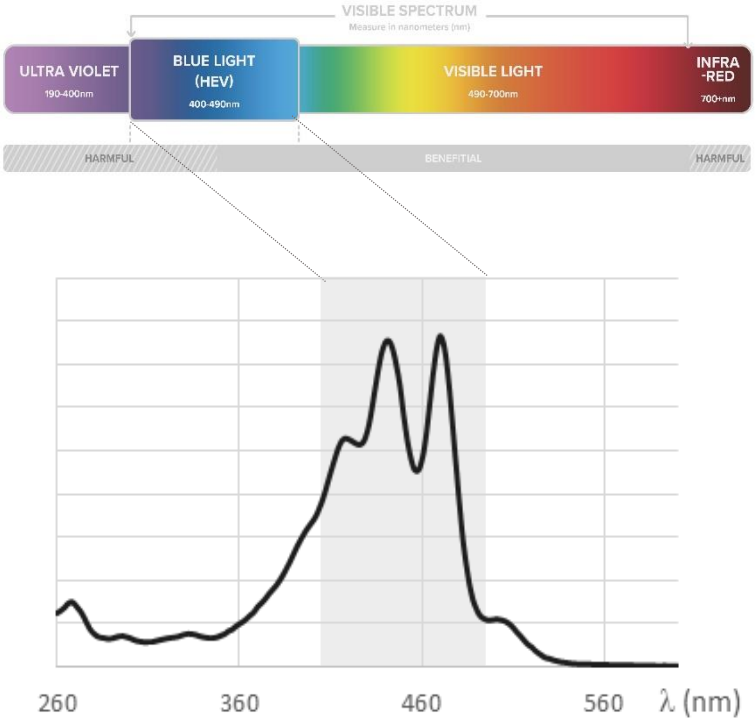
Neurosporene

Lycopene

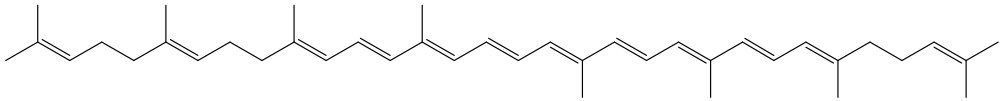
➢ **Greater control of the strain development process ⇒ Faster design**

Neurosporene

Radiance booster, cell energizer and anti-dark spots active ingredient



- ✓ Antioxidant protection
- ✓ Blue light absorption



- Intermediate carotenoid synthesized from phytoene through three-step desaturation reactions with phytoene desaturase
- Red-coloured because of its structure (9 conjugated double bonds that constitute a chromophore) and function (absorptium spectrum)
- Commonly found in plants, bacteria, fungi, algae and serves as a precursor for more than 600 carotenoids[1,2]:
  - *Rhodobacter viridis* train JA737:7 mg/g dry
  - Tomatoes (0.01 mg/100 g^{-1})
    Pink grapefruit (0.38 mg/100 g^{-1})
    Papaya (0.05 mg /100 g^{-1})
- Competition: no commercial product containing neuroposrene on the market

[1]*Biotechnol Lett (2013) 35:1093–1097*
[2]*Khachik et al. 2002*

Neurosporene
Intellectual Property

Marketing

- **FTO**

Free to operate for a method of production of neurosporene from *D. geothermalis* or any other recombinant host cells expressing variant of *crtI* genes encoding the phytoene desaturase and wherein said variant exhibits a modified product specificity towards neurosporene.

- **Patents**

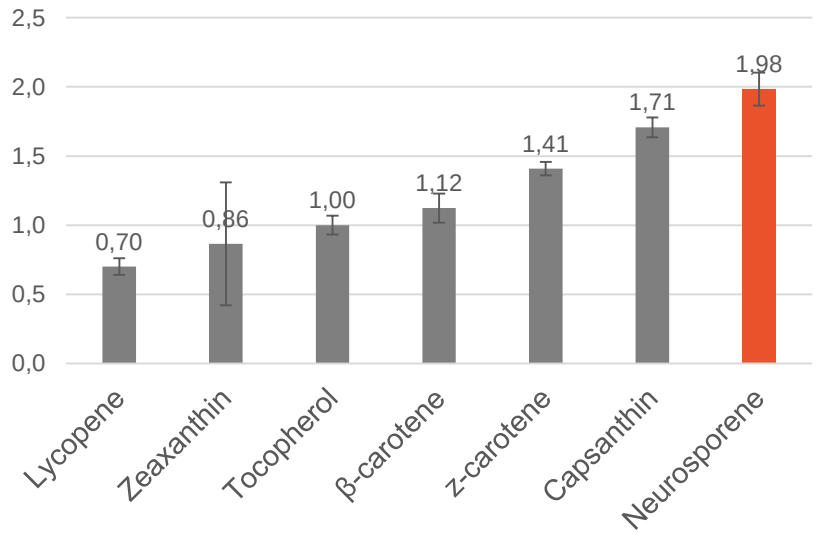
One DEINOVE's patent applications filed that cover DEINOVE's activities on neurosporene applications:

« Utilisation du neurosporène pour protéger la peau des effets délétères de la lumière bleue » (PF29)

Neurosporene
Sharp antioxidant properties

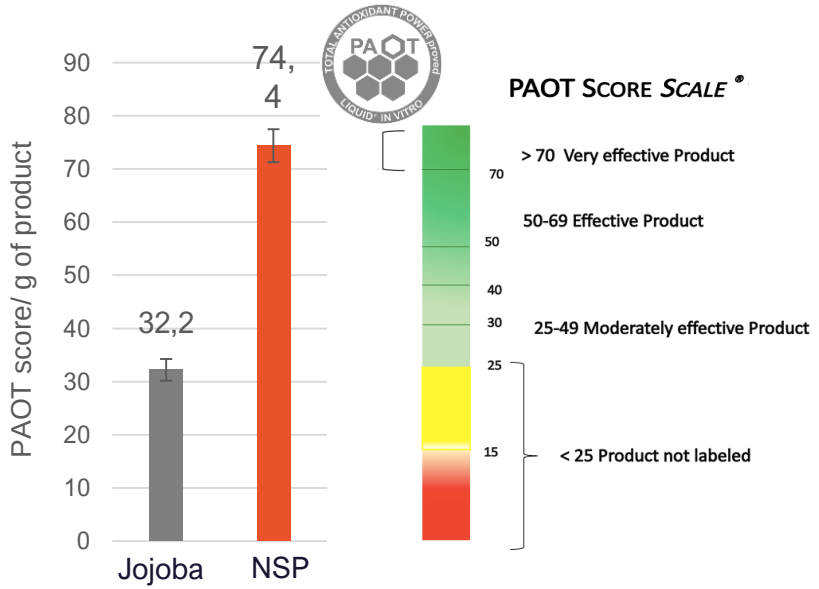
Efficacy

ABTS method



- **Better antioxidant capacity** of neurosporene than well-known antioxidants such as Vitamin E, ß-Carotene, Lycopene, Zeaxanthin, Capsanthin

PAOT Liquid Technology®



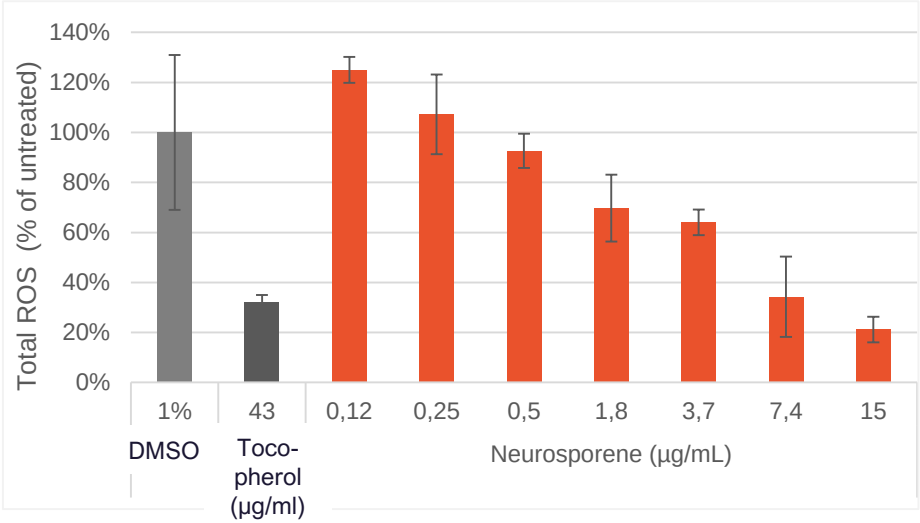
- Classified as a **"very effective product"** in terms of total antioxidant power, which places neurosporene at the top of products scale (Green zone).

Neurosporene
UVA and blue-light protection

Efficacy

- **ROS production under UVA irradiations**

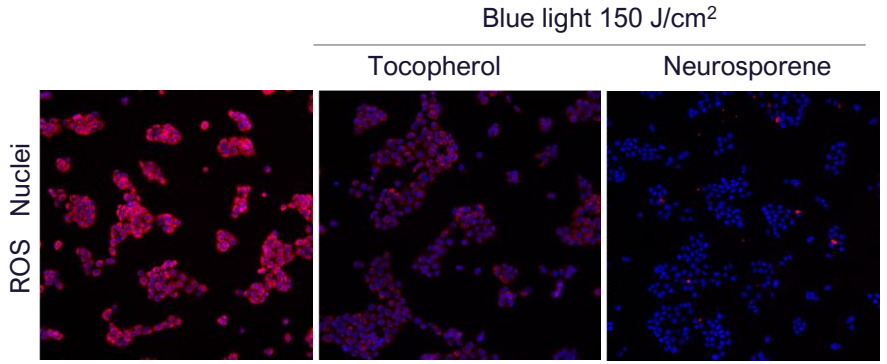
Human keratinocytes; UVA irradiation: 20J/cm²



➢ **Reduction of UVA-induced ROS production** by neurosporene: -80%

- **ROS production under blue light irradiations**

Human keratinocytes; blue-light irradiation: 150J/cm²

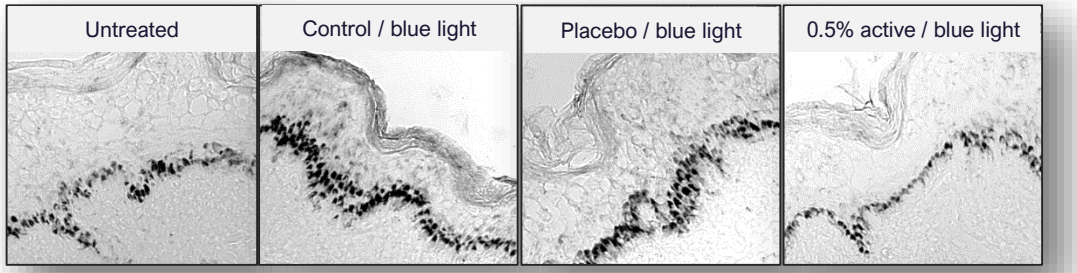


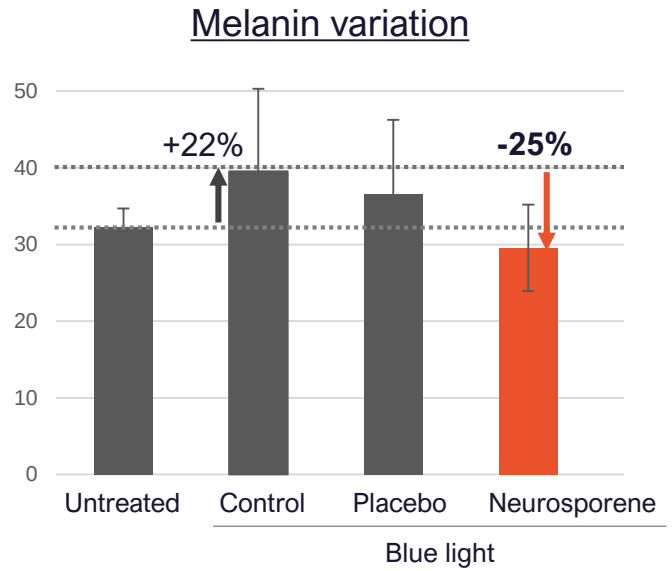
➢ **Inhibition of blue light-induced ROS** by neurosporene: -99%. Activity also observed at the mitochondrial level.

Neurosporene
Sharp antioxidant properties

Efficacy

Melanin staining (Fontana Masson) and quantification on skin explants
Topical application of 0.5% active / blue-light irradiation: 202.6 J/cm² (eq. 6h/day for 8.6 months)





→ Blue light increases melanin synthesis (+22% compared to untreated control) and deposition responsible for the formation of dark spots.

➢ **Reduction of melanin synthesis** in blue light-exposed skin by neurosporene: -25%

Neurosporene

Radiance booster, cell energizer and anti-dark spots active ingredient

Prototype	• Sustainable sourcing: extremophile microorganism collected from a water source in Pyrenees • Strain optimization to get a stable high yield phytoene producing strain in reactor • Fermentation of natural sugars by *Deinococcus*, extraction, purification and formulation in jojoba oil
Industrial process	• Upstream process (fermentation) validation to the 20L scale
Efficacy	• Highly efficient at low dosage (0.5%) for skin protection and radiance enhancer • Outstanding antioxidant with performances that match or exceed leading standards (vitamin E) • UVA & blue-light protection and hyperpigmentation prevention
Innocuity	• Safety confirmed with 100% of the cosmetic ingredient (0.25% neurosporene): Skin irritation (SkinEthic), skin sensitization (Sens-Is® assay delayed in october), ocular irritation (Het-Cam), skin compatibility (Patch test), reverse mutation assay in bacteria (Ames test)
Marketing	• Original storytelling: *Deinococcus,* an extraordinary microorganism famous for its extreme resistance • 1 patent application filed covering DEINOVE's activities on neurosporene applications • 100% Made in France, 100% natural origin (ISO 16128), validated in cosmetic products

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Micrococcus luteus extract

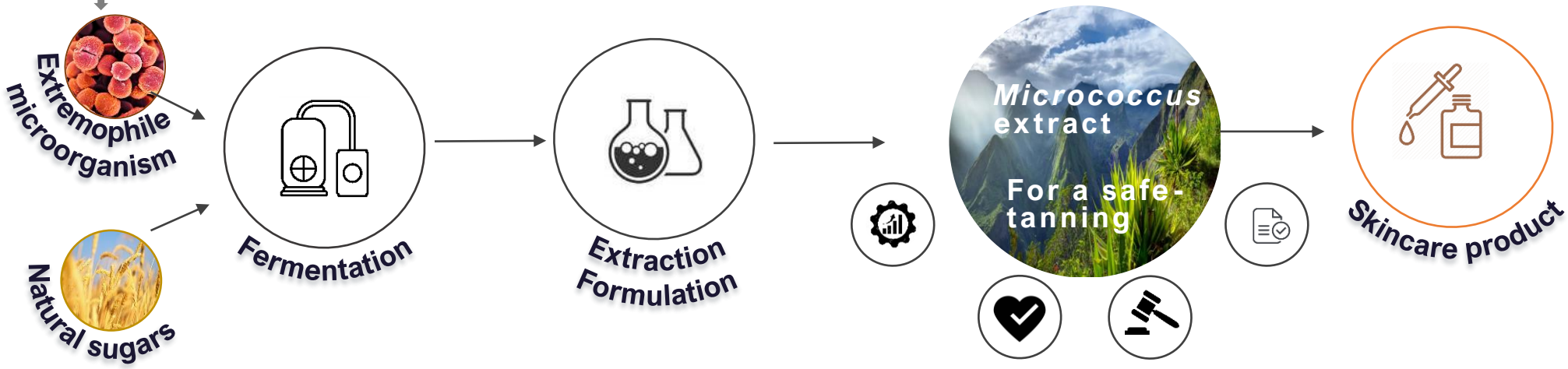
Tropicalis

Micrococcus luteus extract

A sustainable and fully traceable process

Industrial process

- Microorganism collected in the « Manouilh » spring / Salazie cirque / Reunion Island
- At the heart of the Reunion island National park, registered on the UNESCO World Heritage
- Iron-rich water springs surrounded by colorful ochre deposits



- ✓ Identified markers: sarcinaxanthin derivatives
- ✓ Low carbon footprint
- ✓ Independent of seasonality and climate

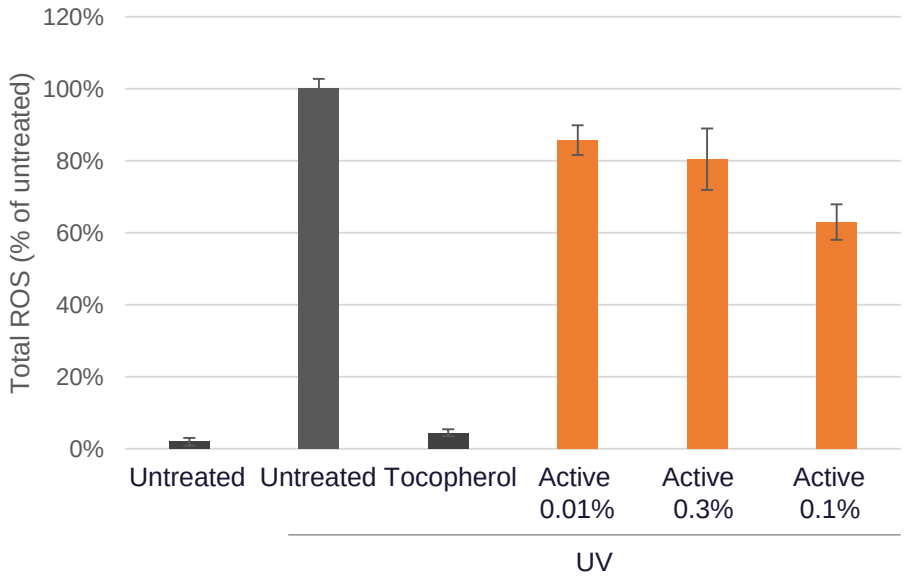
- ✓ No preservatives
- ✓ 100% Made in France
- ✓ Cosmos-compliant

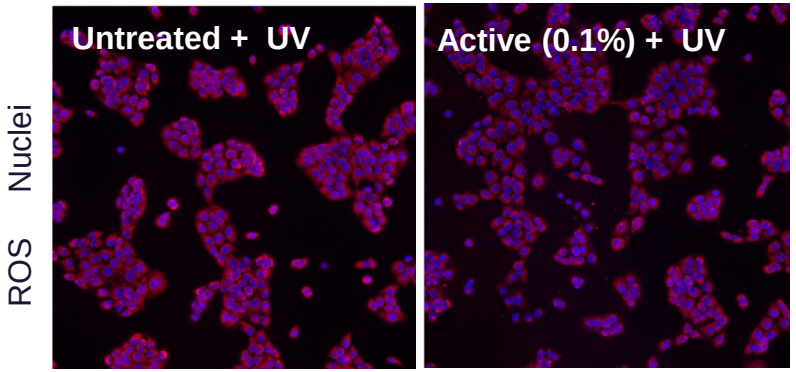
Micrococcus luteus extract
UVA protection

Efficacy

- **ROS production under UVA irradiations**

Human keratinocytes; UVA irradiation: 20J/cm²





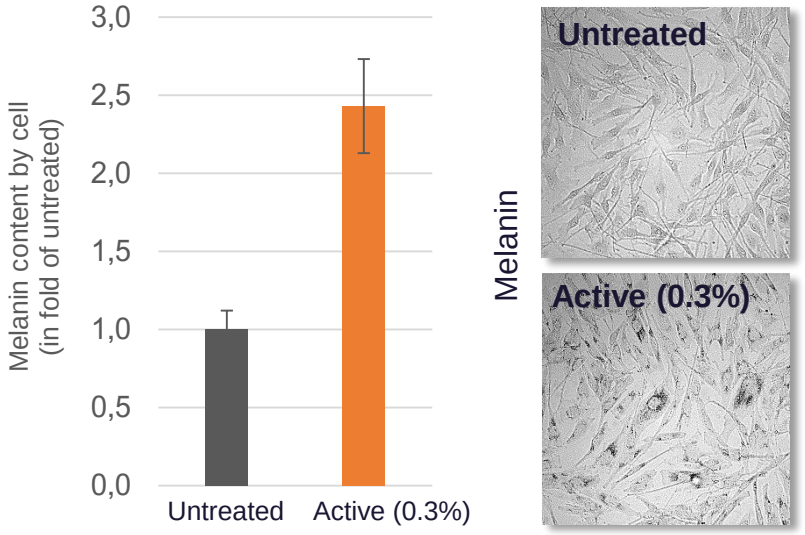
➢ **Reduction of UVA-induced oxidative stress** by *Micrococcus luteus* extract thus protecting skin from deleterious effects of UV

Micrococcus luteus extract
Tanning properties

Efficacy

- ***In vitro* melanin quantification**

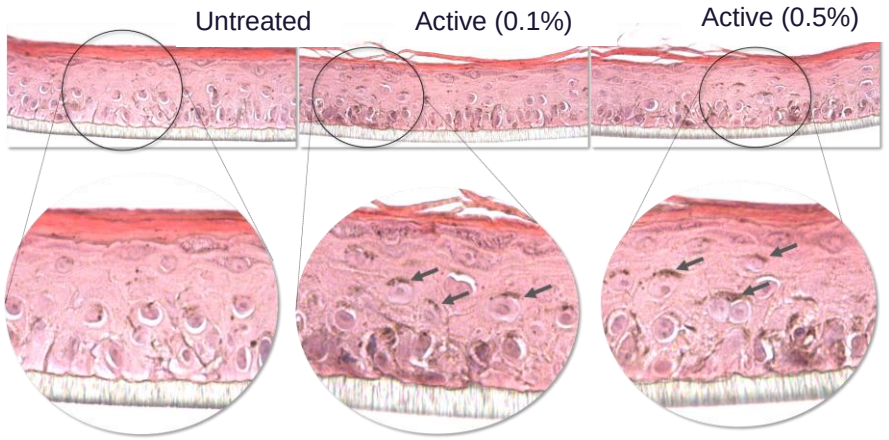
Normal Human Epidermal Melanocytes



➢ **Increase of melanin content** by *Micrococcus luteus* extract: +143%

- ***In vitro* melanin production**

Reconstructed Human Melanized Epidermis

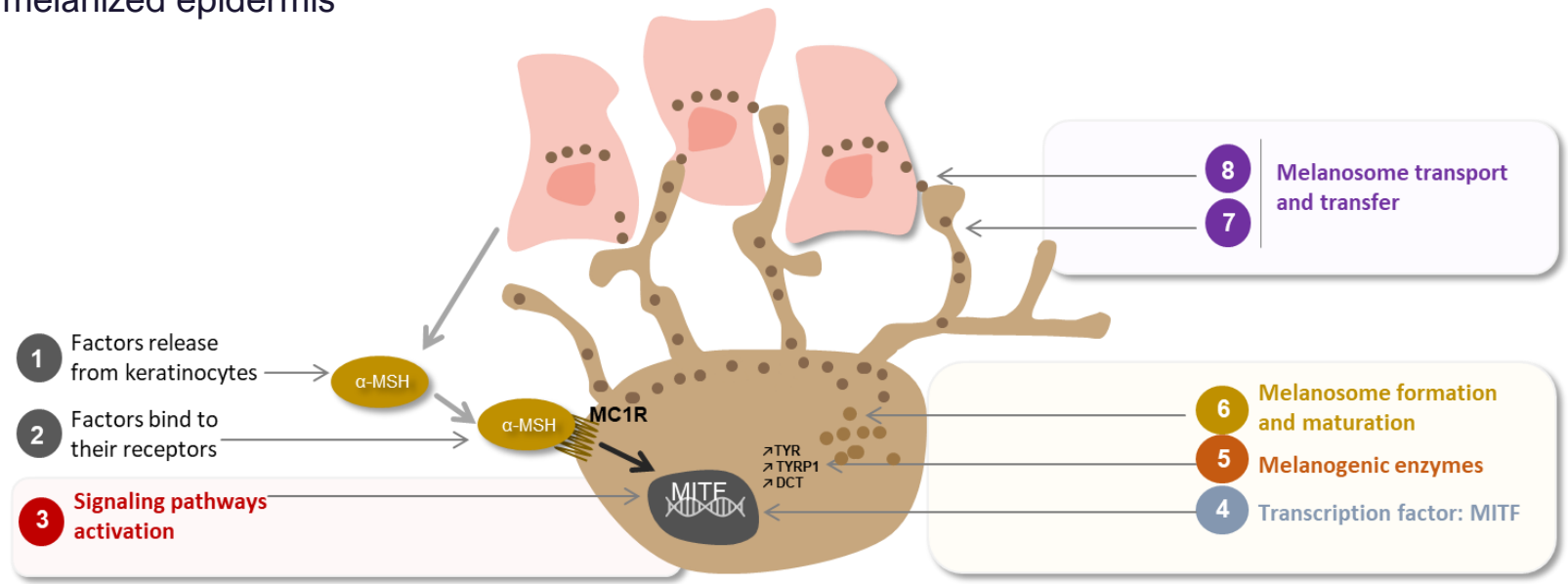


➢ **Visible pigmentation** (melanin domes) induced by *Micrococcus luteus* extract, even at a 0.1%

Micrococcus luteus extract
Putative mode of action

Efficacy

Transcriptomic study
Analysis of key genes involved pigmentation and modulated by the active: 0.1%/0.5% on reconstructed human melanized epidermis



- 14 out of 93 genes involved in the skin pigmentation process are significantly, positively expressed by *Micrococcus luteus* extract.

Micrococcus luteus extract

Photoprotector and natural self-tanning active ingredient

Prototype	• Wild strain taken from a sample of water in Reunion Island • *Micrococcus luteus* is known to produce γ-Cyclic sarcinaxanthin (C50) and its two glycosylated forms[1] • Sustainable and fully traceable process: fermentation of natural sugars by *Micrococcus luteus*
Industrial process	• Process ready for industrial transfer (process book available)
Efficacy	• Dual photoprotection: Antioxidant + tanning properties • Protection from deleterious effects of UV and activation of melanin synthesis • Enhancement of gene expressions involved in different steps of melanogenesis
Innocuity	• Safety confirmed with 100% *Micrococcus* extract: skin irritation (SkinEthic), ocular irritation, skin compatibility (Patch test), reverse mutation assay in bacteria (Ames test), photoirritation and photosensitization
Marketing	• *Micrococcus* strain, UV-resistant bacterium, collected in the heart of the Reunion island National park • 100% Made in France, 100% natural origin (ISO 16128), COSMOS-compliant • Data available on mode of action

[1] *J Bacteriol. 2010 Nov;192(21):5688-99. Netzer*

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Thank you