Healing the downs of psycho-dermatology

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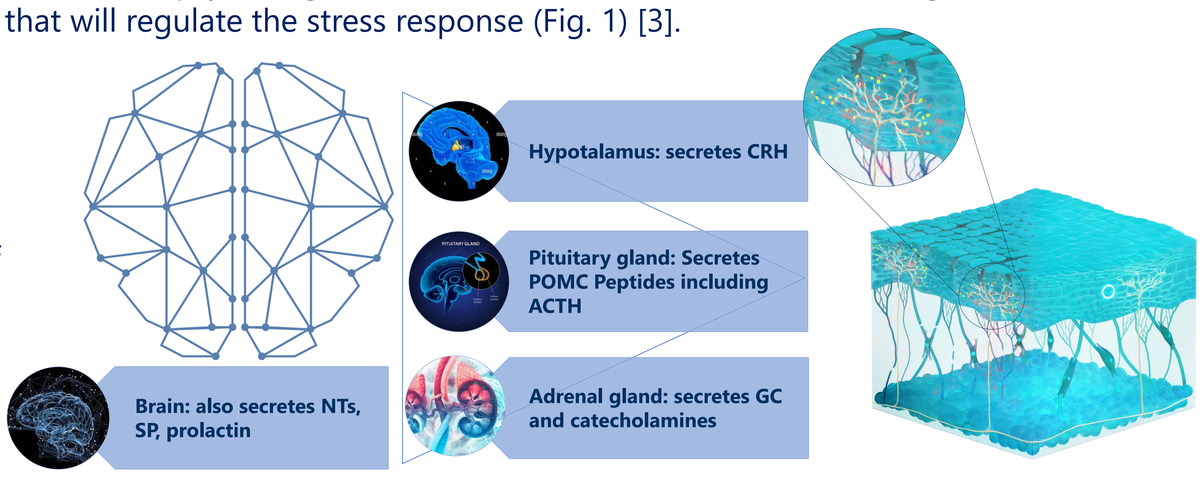
Introduction:

Telomeres are the DNA-based caps and protein structures located at the end of chromosome tips of eukaryotic cells and are important for cell replication during the lifespan. Telomeres shorten with mitosis, [1] and telomere length is a marker of cellular aging [2]. Psychological Stress (PS) has emerged as a main influence on telomere detriment that can trigger aging mechanisms. The crosstalk between the brain and the skin, under psychological stress (PS), activates HPA axis, among others, that stimulates the release of hormones and neuropeptides, like cortisol and substance P, respectively, that will regulate the stress response (Fig. 1) [3].

Chronic exposure to cortisol is related to shorter telomeres length, late wound healing, reduction of epidermal proliferation and differentiation, impaired permeability barrier, decreased integrity of stratum corneum, diminution of extracellular matrix protein, etc [4]. Considering that PS is a common human condition and everyone experiences it at some point in their lives, the development of novel active ingredients in the field of psycho-cosmetics is needed. Due to this, the aim of this study was evaluating the effect of a Micrococcus ferment lysate (BML_72-4), obtained from a sea breeze near Tonga and selected after an extensive screening done by the Spanish National Cancer Research Centre (CNIO), on the triad among emotional stress-telomere length-skin aging.



Materials & Methods:

The microorganism was sampled from ocean aerosol layers at international waters near Tonga, identified by the Whole Genome Sequencing (WGS) and its ferment characterized by metabolomic studies. The effect of the ferment on the telomere length (Telomeric Repeat Binding Factor 1 (TRF1)) and on DNA protection (53BP1) were evaluated by immunofluorescence. The ability of the ferment to recover telomere length was evaluated, by a Dual Quantification qPCR on old vs. young donor fibroblasts. The effect of BML_72-4 on the PS response was analysed in a stressed model of dermis and epidermis where the protein levels of cortisol (ELISA), the nuclear translocation of cortisol receptor (Immunofluorescence) and wound healing (Optical microscopy) were measured. Additionally, the effect of BML_72-4 on cortisol-treated skin explants was evaluated by immunohistochemistry and immunofluorescence. *In vivo* study was performed on 20 Caucasian female volunteers (35-50 years old) showing familiar or work stress and clinical signs of skin aging. A cream with 2% of the ferment was applied twice a day (half-face method), for 28 days and compared vs placebo. Wrinkles depth, age-decrease, body skin hydration, firmness and elasticity were analysed. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

Results & Discussion:

In vitro — Telomere Length Protection

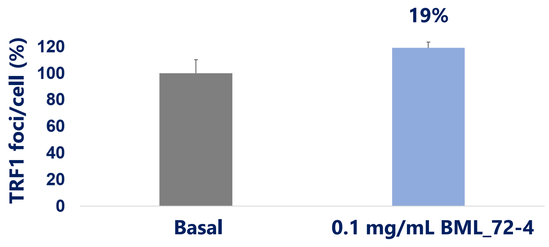
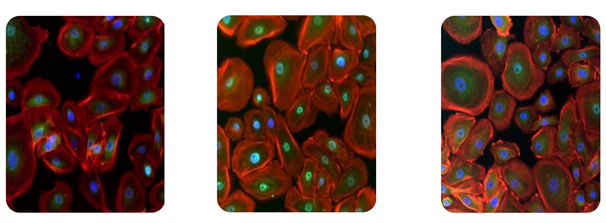


Fig. 2 Levels of Telomeric Repeat Binding Factor (TRF1) in Human Epidermal Keratinocytes (HEKA) treated with BML_72-4 for 24 h.

BML_72-4 increased the levels of **TRF1, a key component of the shelterin complex** which is **essential for telomere protection** (Fig. 2)

Cortisol Receptor Translocation



-20%** of NR3C1 **translocation** vs cortisol

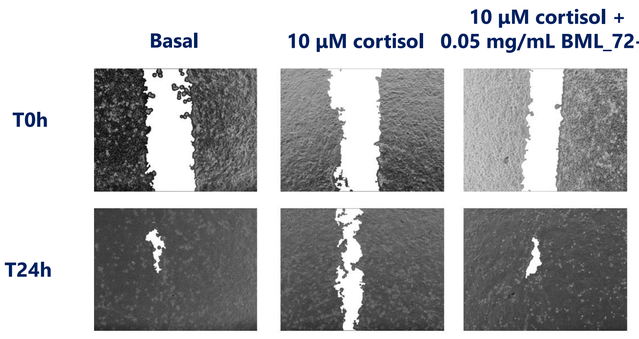
Fig. 3 Nuclear Glucocorticoids Receptor (NR3C1) fluorescence images on HEKa after 24 h treatment. Images acquired with a fluorescence microscope (green: NR3C1, red: phalloidin, blue: nuclei).

PS stimulates the **continuous release** of cortisol and substance P which will lead to telomeres detriment and other skin concerns proving that **skin acts** as an **endocrine organ** [3;5].

BML-72-4 → **-18%** cortisol release; **-68%*** **DNA damage** marker (53BP1)

BML_72-4, on stressed cell models, **reduces** the **levels** of **cortisol (-18%**)** (results not show) and the **nuclear translocation** of **NR3C1** (cortisol receptor) (Fig. 3).

Migration Recovery of Stressed HaCaT Cells



+37%* of regeneration** vs stress condition

Fig. 4 10X phase-contrast images of migrating HaCaT after 24 hours of treatment.

The **ferment increases** the levels of proteins which synthesis are affected by the increase of cortisol or substance P such as **collagen, elastin** and **KRTs** [6] (results not shown). In addition, the **ferment restores** the **ability** of **human keratinocytes** to **regenerate** under **stress conditions** (Fig.4).

Ex vivo — Stratum Corneum Integrity

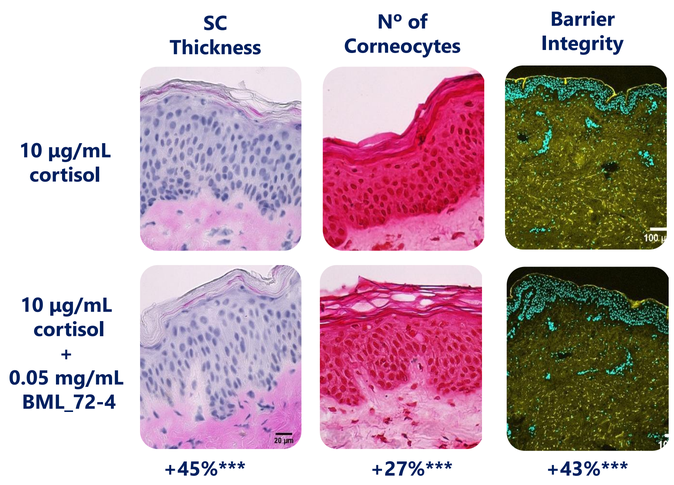
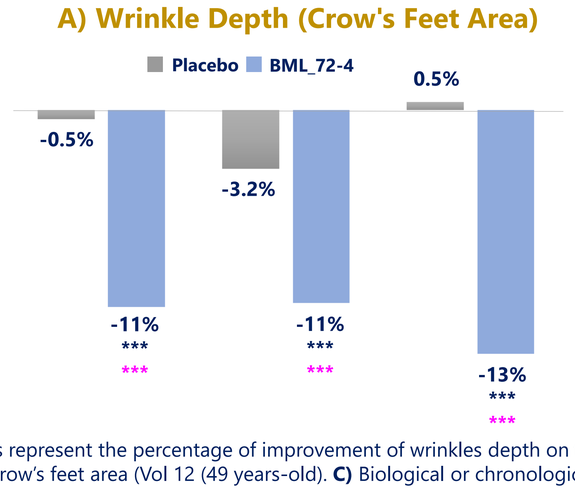


Fig. 5 Images of Stratum Corneum (SC) thickness, corneocyte layers and barrier integrity in skin explants where the SC was previously removed. After removal of SC, the explants were treated every 24 h during 5 days. Cryofixed sections were stained with Haematoxylin and Eosin, 1% Safranin-O red solution and Lucifer yellow, respectively. The images were obtained with light and fluorescence microscopes.

BML_72-4 protects the **skin** from **decreased SC thickness,** a **reduction** in the **number** of **corneocyte layers** and **deterioration** of skin **barrier integrity** (Fig. 5)

These results suggest a potential role of BML_72-4 to modulate cortisol-substance P-stress response.

In vivo — A) Wrinkle Depth (Crow's Feet Area)



B) Anti-Wrinkle Effect (Crow's Feet Area)

C) Biological Age Effect

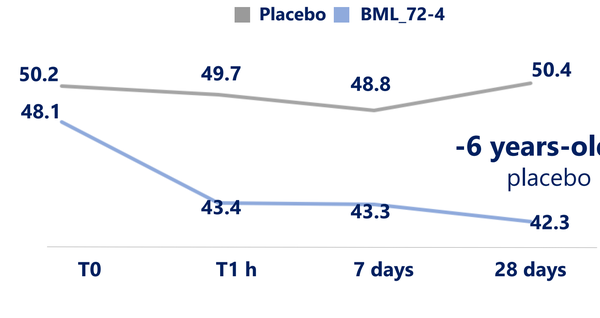


Fig. 6 A) Bar Graphs represent the percentage of improvement of wrinkles depth on the crow's feet area (Primos 3D) (*Blue vs T0 and pink *vs* placebo). **B)** VISIA® Illustrative images of the wrinkles in the crow's feet area (Vol 12 (49 years-old). **C)** Biological or chronological age evolution.

As the skin is an organ of perception, the internal disturbance promoted by PS generates multiple skin problems [7] that the **active ingredient** could counteract, **increasing** the **skin hydration (+39%***), firmness (+9%***)** and **elasticity (+8%***)** (results not shown) and **reducing** the **depth** of **wrinkles** (Fig.6A and B) and **perceived age** (Fig.6C) on **stressed volunteers.**

Conclusions:

Psychological stress can be linked with skin aging since it negatively affects telomere length and epidermal/dermal functions, thereby accelerating the natural senescence process. According to our efficacy studies, the bacterial ferment shown here (BML_72-4) is capable of restoring "skin allostasis" by reducing the effect of stress neuroendocrine response promoting the healing of the downs of psycho-dermatology.

References:

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