Skin proteome protection through the chaperone-like activity of an *Arthrobacter agilis* extract.

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BACKGROUND

The proteome is the set of proteins present in the organism at a given time and these proteins play major roles to allow the proper functioning of the cells, the tissues and the organisms. For instance, extracellular proteins (eg collagen, elastin) have structural roles allowing cohesion and elasticity of the skin, other proteins protect the tissue against pathogens or oxidative damages while another group of proteins repair the DNA in case of lesions. **Therefore, maintaining a healthy proteome is at high stake for the skin to keep a proper structure and function.**

To be able to assume their roles, proteins need to acquire and maintain their structure, which can be affected by stress such as oxidative damage or heat shock. One of the main damage of proteins is **carbonylation**: this non-enzymatic posttranslational modification can affect the complete set of proteins and leads to their loss of structure and function in a short-term effect. In the long term, non-degraded carbonylated proteins may form aggregates that can have deleterious effect on the cells and tissues. Fortunately, a specific family of proteins called chaperones helps other proteins to fold properly and protects them against unfolding, sensitivity to carbonylation and loss of functionality. **We discovered and characterized a bacterial extract from *Arthrobacter agilis* (SBE) that displays a chaperone-like activity and protects the skin proteome against carbonylation.**

MATERIAL & METHODS

- Extract preparation and characterization

SBE was obtained from *Arthrobacter agilis* through an alcoholic extraction (ethanol + ethyl acetate). It is composed of 6 bacterioruberins as observed by HPLC. SBE is stabilized in caprylyl/capric triglyceride and tocopherols.

- SBE binding and chaperone activity

- Binding to BSA: BSA (1,6mg/ml in 25% DMSO) alone or BSA + SBE (in 25% DMSO) were injected in a separation column (Shodex protein KW-803) in 50mM Nah_2PO_4 + 300mM NaCl, pH 7 in a constant flux (0,8ml/min). HPLC profile at 220nm was studied.
- To assess chaperone activity, SBE or control were added at 4µM to an Alkaline phosphatase (AP) solution (10µl of 10^{-5}) and the mixture was incubated at 37°C under slow agitation prior to a heat shock (55°C, 1hr). Finally, 50µl of AP-substrate was added and the kinetic of enzyme activity was red at 405nm.

- Protein carbonylation in cells

Normal Human Epidermal Keratinocytes were grown in SFM. 1 day after plating, cells were treated with SBE in DMSO. Next day cells were irradiated with 28J/cm² UVA for 5min, 72J/cm² blue light for 20min or incubated with 50µg/ml of fine particles (PM_{10}) for 24hrs. After the stress, carbonyl groups were labelled using a specific probe and total proteins with cy5 NHS. Ratio of carbonylated on total proteins was quantified.

- Elastin aggregation :

Soluble elastin was subjected to UV irradiation (5J) or osmotic stress (NaCl 0,4M) in presence or absence of SBE. The aggregation of elastin was then measured by the absorption at 400nm, the increase correlating to the light scattering of aggregated elastin.

- Protein carbonylation and elastin degradation in explant.

SBE were topically applied on skin explant for 4 days. On day 5, explants were washed and irradiated with UVA (6J/cm²) and incubated with fine particles (PM_{10}, 1mg/ml). Histological sections were labelled with a probe for carbonylated proteins or antibodies directed against elastin and DAPI for DNA.

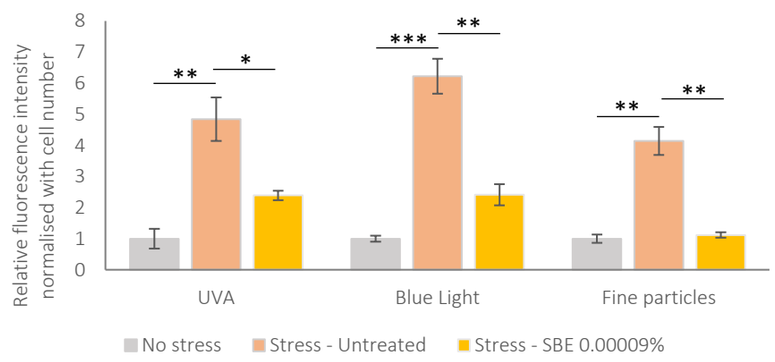
- Statistical analysis

Student t-tests were performed to compare experimental groups. The results are reported on the figures, *:p<0.05, **:p<0.01 and ***:p<0.001, n.s. not significant.

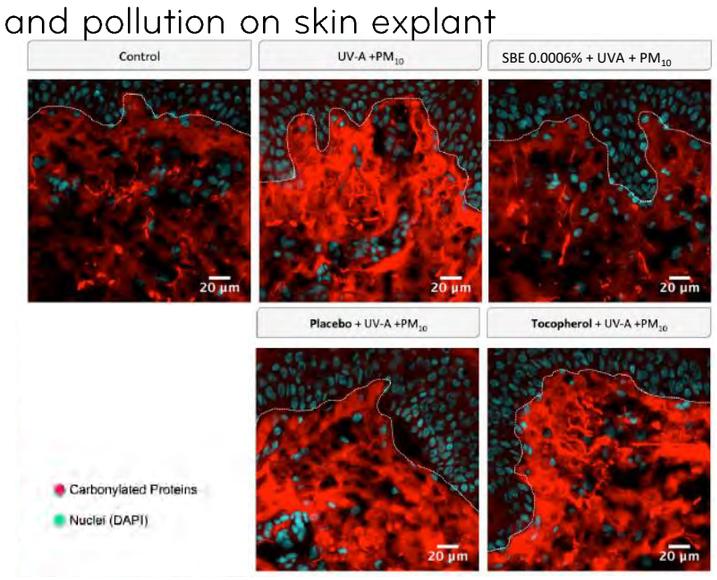
RESULTS

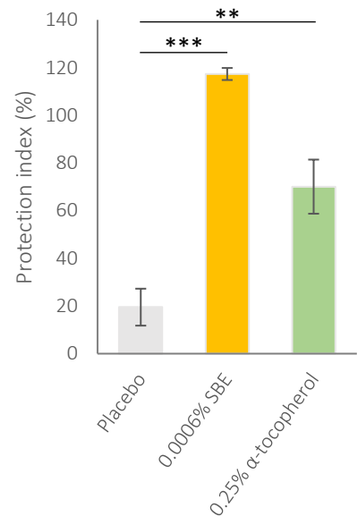
1. SBE binds to proteins and displays chaperone-like activity

2. SBE protects NHEK against carbonylation induced by various stresses

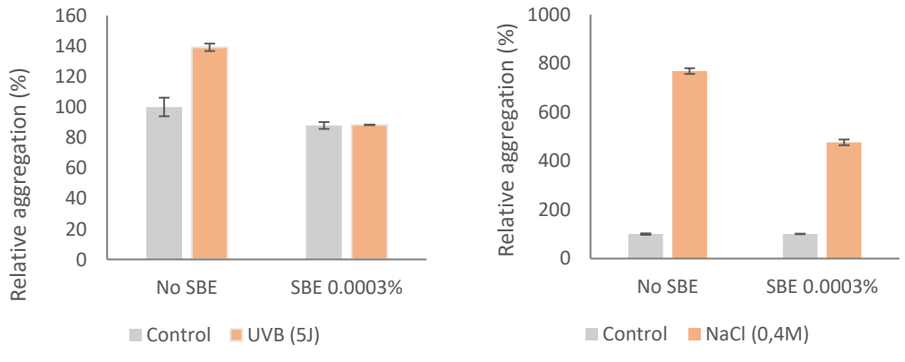


3. SBE protects proteins against carbonylation induced by UV and pollution on skin explant





4. SBE protects elastin from stress-induced aggregation *in tubo*



5. SBE protects elastin against degradation induced by UV and pollution on skin explant

CONCLUSION

Our results show that SBE can bind to proteins and possesses a **chaperone-like activity**. This activity allows a significant protection of the proteome against carbonylation, aggregation and degradation induced by stresses originating from the environment. **All together, *Arthrobacter agilis* extract is of great interest for proteome protection and can be an innovative ally to fight against the wide variety of everyday life stresses that leads to premature aging of our skin.**

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