“Anti-Aging”: Exploiting the Greek microbial diversity for the discovery and development of novel antiaging molecules.

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Abstract

Greek ecosystems are a fertile, yet under-explored ground for the study of Actinobacteria, known producers of bioactive compounds. By exploring this under-investigated biodiversity and chemodiversity of Greek actinobacterial strains (mostly of *Streptomyces* sp.), the “Anti-Aging” project aims to uncover potentially novel natural compounds with anti-aging activity, that can be formulated as cosmeceutical products. In total, 1000 isolates belonging to the Athens University Bacterial & Archaea Culture Collection (ATHUBA) were studied. A customized in-house library of ~2000 extracts was generated (EtOAc and MeOH/H_2O), all of which were investigated for potential anti-aging properties. This biological evaluation was performed though elastase (anti-aging activity) and tyrosinase (whitening activity) inhibition *in vitro* assays. The biological properties of ~100 non-cytotoxic and active extracts were also tested on human diploid fibroblasts (BJ) for their anti-aging activity, and on melanocytes (B16F10) for their whitening activity. Fractions (~120) of the most active, non-toxic extracts tested for elastase and tyrosinase inhibition activities in cell-free and cell-based *in vitro* assays. The most promising highly active fractions were subjected to compound isolation and bioactivity assessment. Isolated compounds tested for their ability to activate cyto-protective mechanisms in cell-based assays, while their anti-aging effects also tested in *in vivo Drosophila melanogaster* models, for their ability to induce antioxidant mechanisms through activation of the Nrf2/cncC transcriptional factor. Our promising results show a hidden, yet powerful potential of harnessing Greek microbial wealth, in the context of anti-aging.

Materials and Methods

A unique library of 2000 extracts (EtOAc (.1) and MeOH/H_2O (.2)) was generated, and their anti-aging properties were evaluated via enzymatic and cell-based assays. Extracts with tyrosinase and/or elastase inhibition properties (Figure 2), were chosen for cytotoxicity tests (MTT assay), in BJ (human fibroblasts) and HaCaT (human keratinocytes) cells. Extracts tested in four different concentrations in each cell line (1, 2, 5, 10 μg/ml) for 24h (Figures 3). B16F10 (melanocytes) and BJ cells were treated with 2 μg/ml of selected non-toxic extracts for 48h, for tyrosinase and elastase inhibition evaluation (Figure 4). Extract M43B.1 selected for fractionation and compound isolation (Figure 1). Six isolated compounds tested in cell-based assays for their anti-ageing (anti-elastase and anti-tyrosinase activity, Figure 5) and cyto-protective potential (antioxidant, proteasome and lysosome activation ability, Figures 6,7,8). Four promising compounds tested in *in vivo* models for the same properties (Figure 9,10), and the most promising one tested for its life-extending ability of *Drosophila melanogaster* flies (Figure 11).

Compounds

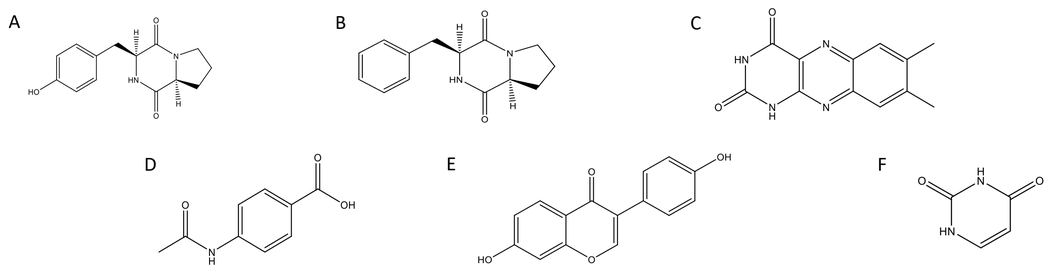


Figure 1. Isolated compounds of extract M43B.1. A) M43B/1/7/13, B) M43B/1/7/5, C) M43B/1/9/1, D) M43B/1/9/3, E) M43B/1/11_12/2 and F) M43B/1/11_12/5.

Results

Extract screening

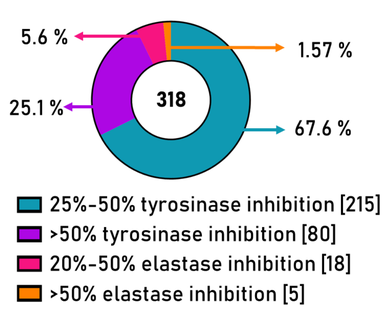


Figure 2. Enzymatic assays reveal 318 active extracts. Most of them exhibit tyrosinase inhibition (295) and fewer elastase inhibition (23).

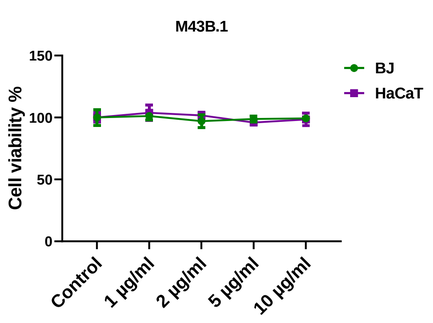


Figure 3. MTT assay. Extract M43B appears to be non-toxic (>90% viability) in both BJ (green) and HaCaT (purple) cells.

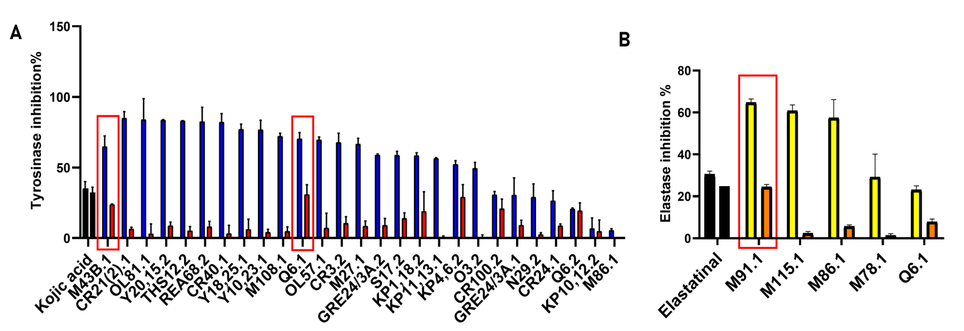


Figure 4. Cell-free and cell-based (A) tyrosinase and (B) elastase inhibition assays. Extracts M43B.1 and Q6.1 exhibit the highest anti-tyrosinase activity, while M91.1 the highest anti-elastase activity. Kojic acid (2 μg/ml, 100 μg/ml) and elastatinal (0,5 μg/ml) were used as positive controls. Blue, Yellow: Cell-free (200 μg/ml). Red, Orange: Cell-based (2 μg/ml).

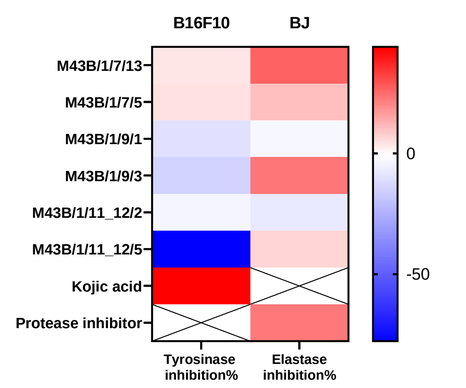


Figure 5. Tyrosinase and elastase inhibition levels (%). B16F10 and BJ cell treated with the compounds (2 μM) for 48h. Kojic acid (100 μg/ml) and protease inhibitor (1X) were used as positive controls.

Cell-based compound evaluation

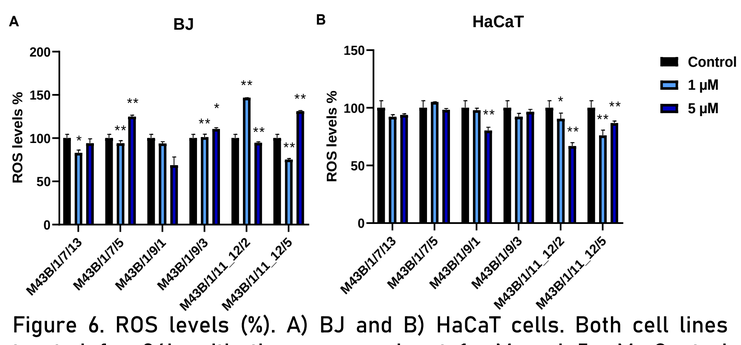


Figure 6. ROS levels (%). A) BJ and B) HaCaT cells. Both cell lines treated for 24h with the compounds at 1 μM and 5 μM. Control samples treated with DMSO. *p<0.05, **p<0.01, +SD.

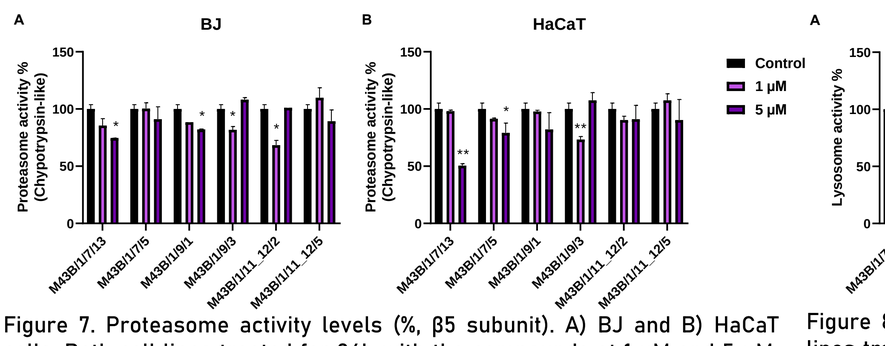


Figure 7. Proteasome activity levels (%, β5 subunit). A) BJ and B) HaCaT cells. Both cell lines treated for 24h with the compounds at 1 μM and 5 μM. Control samples treated with DMSO. *p<0.05, **p<0.01, +SD.

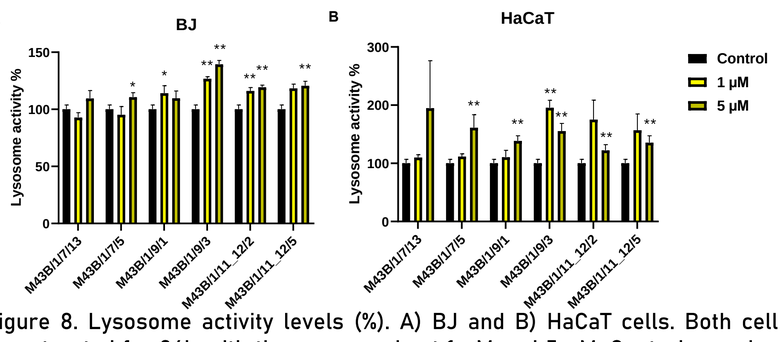


Figure 8. Lysosome activity levels (%). A) BJ and B) HaCaT cells. Both cell lines treated for 24h with the compounds at 1 μM and 5 μM. Control samples treated with DMSO. *p<0.05, **p<0.01, +SD.

In vivo compound evaluation

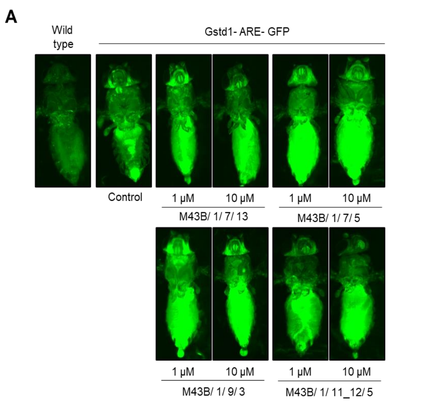


Figure 9. Measurement of compound ability to activate antioxidant response pathways. A) Imaging and B) measurement of GFP levels (%, RFUs/μg protein) of somatic tissues of young (7-10 days) female transgenic GstD1-ARE: GFP flies who were treated for 7 days with 1 μM and 10 μM of the selected compounds. A) Wild type and non-treated GstD1-ARE: GFP flies, treated with the vehicle (DMSO). B) Non-treated GstD1-ARE: GFP values set to 100%. + SD, p**<0.01.

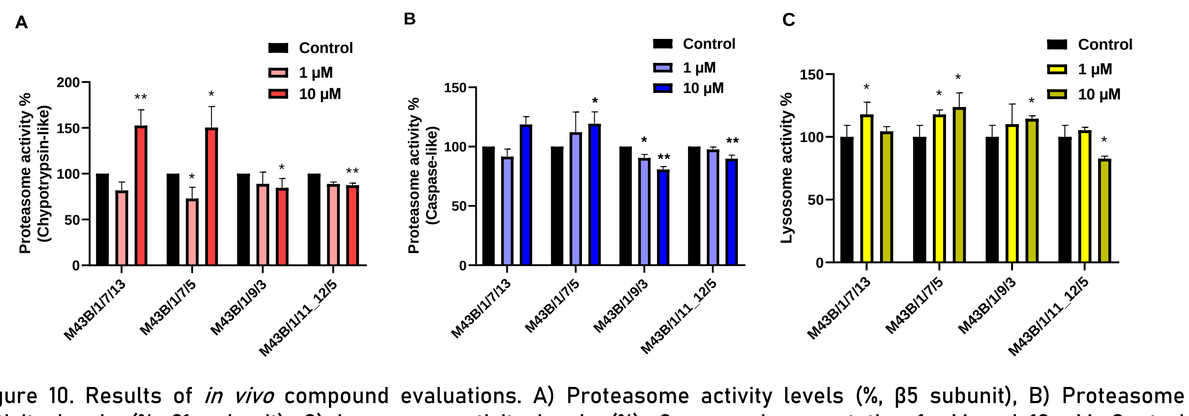


Figure 10. Results of *in vivo* compound evaluations. A) Proteasome activity levels (%, β5 subunit), B) Proteasome activity levels (%, β1 subunit). C) Lysosome activity levels (%). Compound concertation 1 μM and 10 μM. Control population treated with DMSO. *p<0.05, **p<0.01, +SD.

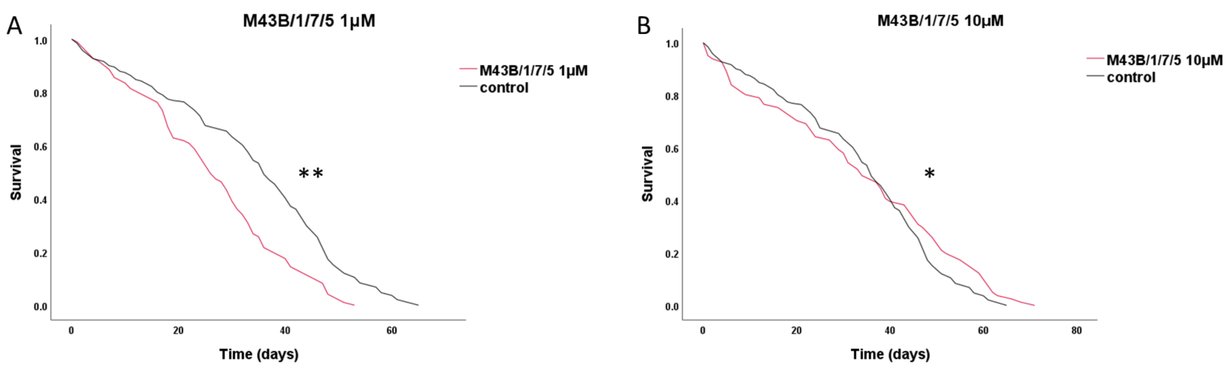


Figure 11. Compound effects on the viability of the flies. Life spans of flies after continuous treatment in A) 1 μM and B) 10 μM of the compound M43B/1/7/5. Control populations were treated with DMSO, p*<0.05, p**<0.01.

Conclusions

The Anti-Aging project aims to identify molecules with antiaging potential that can be formulated as novel cosmeceutical products. Our biological assessments, reveal known molecules with anti-aging activity. To sum up:

- A high number of Actinobacteria extracts exhibit anti-aging properties, like tyrosinase and elastase inhibition.
- The EtOAc extract M43B is highly active in both cell-free and cell-based assays.
- Isolated compounds of M43B activate lysosomic pathways in cell-based assays in both BJ and HaCaT cells.
- In *in vivo* assays, all compounds activate antioxidant pathways through the Nrf2/cncC transcriptional factor.
- Compound M43B/1/7/5 is the most promising, since it can activate both the proteasomic and lysosomic activities, while in 10 μM it is able to improve the survival rate of flies after 40 days.

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