Exploiting the Greek microbial diversity for the discovery and development of novel antiaging molecules

HELLENIC REPUBLIC
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Introduction

Actinobacteria from Greek ecosystems are renowned for producing bioactive compounds. To this purpose, the "AntiAging" project sought to uncover new, promising natural compounds with anti-aging properties, which could be incorporated into cosmeceutical/nutraceutical formulations. Herein, 1000 isolates originating from the Greek natural environment and belonging to the ATHUBA collection (Athens University Bacterial & Archaea Culture Collection), were investigated.

Materials & Methods

A unique library of ~2000 extracts (EtOAc and MeOH/H_2O) was generated, and the tyrosinase and elastase inhibition activity was evaluated via enzymatic assays. An ultrasound-assisted extraction with acetone was used to achieve cell lysis. Acetone was then evaporated, and cultivation broths were extracted 2 times with EtOAc and 2 times with MeOH **(Figure 1)**. Obtained extracts were evaporated using a centrifugal evaporator and resuspended in DMSO for the assessment of bioactivity.

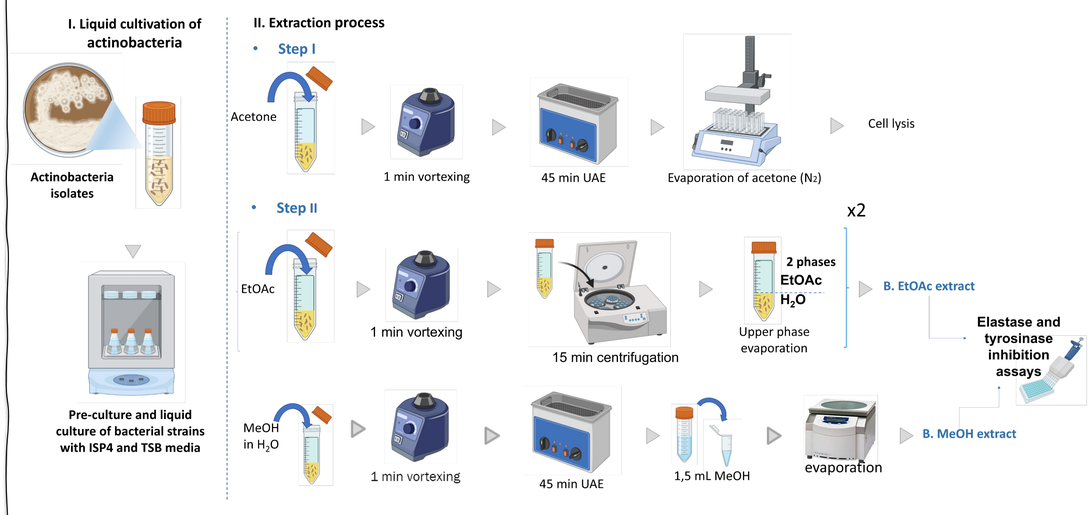

Figure 1. Extraction protocol followed to generate the in-house custom library of ~2000 extracts .

Results

The screening data demonstrated that 1.5% of strains produced elastase inhibitors and 26.5% produced tyrosinase inhibitors. Ethyl acetate appears to be more efficient solvent for extraction of molecules of interest. Out of the top 100 extracts with the highest inhibitory activity, 30 were found to be non-cytotoxic, when tested on cell-based assays. The top 4 microbial strains, belonging to *Amycolatopsis* sp. and *Streptomyces* sp. families, combining a high production of secondary metabolites and bioactivity on both cell-free and cell-based assays, with inhibition rates ranging from 35-65% and 20-29% **(Table 1)**, respectively, were subjected to a scale-up process (2L) and fractionation using HPLC-DAD-ELSD. The bioactivity of each fraction was further assessed via enzymatic assays **(Figure 2)** and the most active fractions are being analyzed by UPLC-HRMS using a high-throughput dereplication and molecular networking method **(Figures 3, 4 & 5)**. To meet the requirements of the isolation procedure, the microbial strain M43B was subjected to an 8L cultivation, extracted with EtOAc and fractionated using Sephadex® LH-20. The purified molecules were characterized by an extensive analysis of NMR and HRMS spectra data and will be further evaluated for their antioxidant activity, the proteasome activation and their impact on longevity of in vivo models (*Drosophilla* sp.) **(Figure 6)**.

Extract	Cell-free tyrosinase inhibition %	SD	Cell-free elastase inhibition %	SD	Cell-based tyrosinase inhibition %	SD	Cell-based elastase inhibition %	SD
M43B	**64,788**	7,535	-	-	**23,817**	0,327	-	-
Q6	**65,678**	7,241	-	-	**20,572**	2,888	-	-
KP4,6	**52,212**	2,623	-	-	**29,015**	8,843	-	-
M91	-	-	**64,708**	1,755	-	-	**24,438**	1,234

Table 1. Cell-free & cell-based tyrosinase/elastase inhibition on the selected 4 extracts.

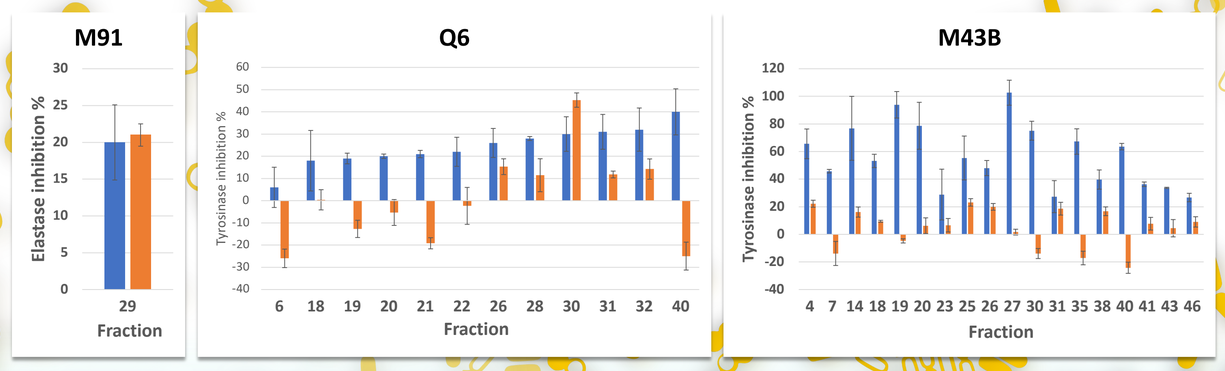

Figure 2. Cell-free & cell-based tyrosinase/elastase inhibition of active fractions of the extracts M91, Q6 and M43B. **Legend:** ■ cell-free; ■ cell-based.

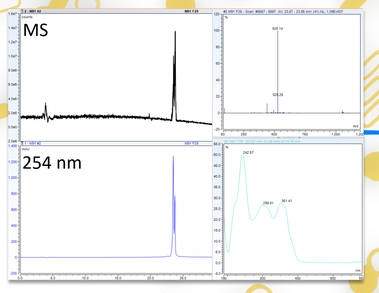

Figure 3. Chromatographic & spectroscopic data from Fraction 29 of M91 extract (*Thermo* Scientific™ *ISQ*™ EC Single Quadrupole Mass Spectrometer).

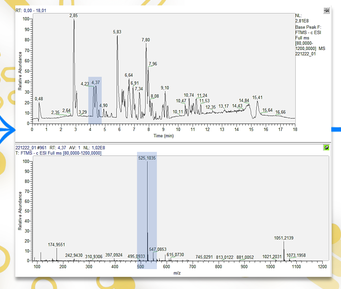

Figure 4. UHPLC-HRMS analysis of the M91 EtOAc extract (Thermo Scientific™ LTQ XL™ linear ion trap mass spectrometer).

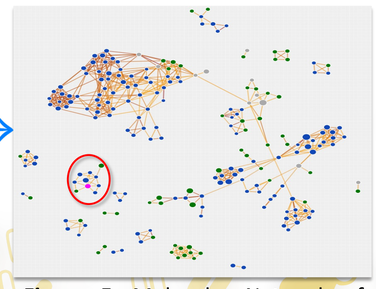

Figure 5. Molecular Network of M91 extract. **Legend:** • MS2 match; • DDA for another ion; • No MS2; • Cluster of interest.

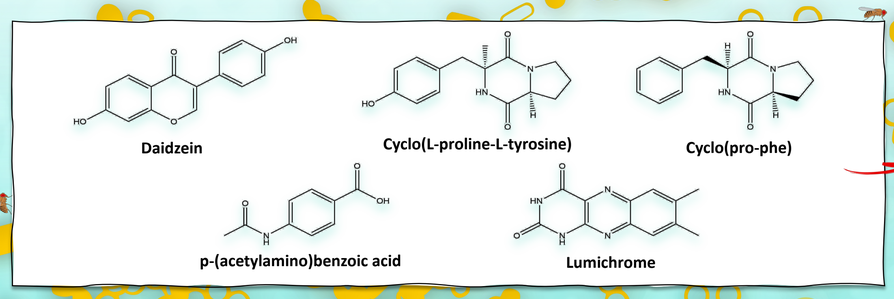

Figure 6. Isolated compounds from the EtOAc extract of M43B characterized by NMR & HRMS spectra.

Longevity of in vivo models

The isolated compounds resulting from the fractions of M43B EtOAc extract will be evaluated in the in vivo experimental model of *Drosophila melanogaster*. Following the administration of the selected metabolites as a dietary supplement, their antioxidant activity will be evaluated at various molecular levels. In addition, the in vivo activation of cellular protective anti-aging systems of the proteasome and autophagy will be assessed. Finally, they will all be evaluated for their potential to increase the longevity of insects (*Drosophilla* sp.) after continuous administration to a large number of experimental animals.

Ongoing work

The fractionation of M43B extract resulted in 14 fractions which were further purified using Sephadex® LH-20 and PREP-TLCs. A total of 20 fractions were analyzed with UPLC-HRMS and dereplication was performed. Compound annotations indicate a strong prospect of isolating promising bioactive molecules which could be integrated into cosmeceutical formulations **(Figure 7)**.

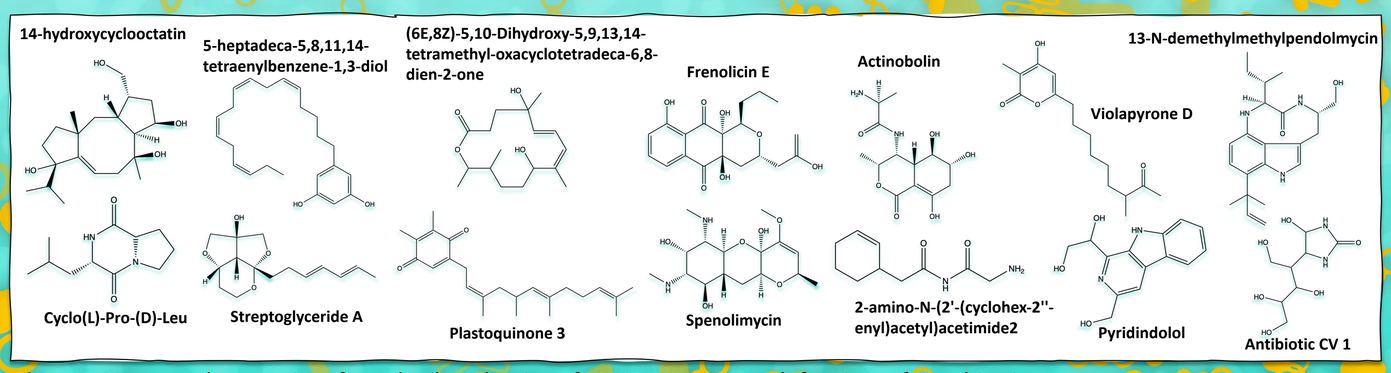

Figure 7. Compound annotations from the dereplication of M43B EtOAc extract's fractions of 8L cultivation.

Conclusion

Our ultimate goal is the targeted identification and isolation of bioactive compounds, which can be integrated into cosmeceutical formulations. The plethora of unidentified molecules highlight the significant potential for exploiting Greek microbial diversity in the realm of anti-aging research.

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