Exploiting the Greek microbial diversity for the discovery of novel anti-aging molecules with applications in cosmeceuticals

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Abstract

Greek ecosystems are a fertile, yet under-explored ground for the study of Actinobacteria which are known producers of bioactive compounds. By exploring the under-investigated biodiversity and chemodiversity of Greek actinobacterial strains (mostly of *Streptomyces* sp.) the "Anti-Aging" project aims to discover novel natural products (NPs) that can be formulated as cosmeceuticals. In total, 1,000 isolates belonging to the National and Kapodistrian University of Athens (NKUA), Greece, Bacterial and Archaea Culture Collection (ATHUBA; some originating from unique environments such as caverns, volcanoes, thermal springs, etc.) were studied. A customized in-house library of 2,000 extracts was generated (EtOAc and MeOH/H_2O) all of which were investigated for potential bioactivity. Biological evaluation was initially performed though elastase (anti-aging activity) and tyrosinase (whitening activity) inhibition cell-free assays. A small number of extracts demonstrated >50% elastase inhibitory activity, whereas a considerably larger number exhibited >50% tyrosinase inhibitory action; in some cases, the bioactivity exceeded 80% inhibition at cell-free assays. Of the top ~100 extracts showing the highest inhibitory activity, 33% were found to be non-cytotoxic when tested on cell-based assays. The biological properties of the non-cytotoxic extracts were tested on human diploid fibroblasts (BJ cells) for their anti-aging activity and on melanocytes (B16F10 cells) for their whitening activity. The most promising isolates were subjected to a scale-up process (2L) and fractionation (~120 fractions) and their bioactivity for elastase and/or tyrosinase inhibition was reassessed; finally, the most active fractions were used for compound identification and isolation. Our promising results reveal 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 and MeOH/H_2O) was generated, and the tyrosinase and elastase inhibition activities were evaluated via enzymatic and cell-based assays (Figure 1). Extracts with tyrosinase and/or elastase inhibition properties (Figure 2), where chosen for cytotoxicity tests (MTT assay), in BJ and HaCaT cells. Extracts tested in four different concentrations in each cell line (1, 2, 5, 10 μg/ml) for 24h (Figures 3, 4). B16F10 and BJ cells were treated with 2 μg/ml of selected non-toxic extracts for 48h, for tyrosinase and elastase inhibition evaluation (Figure 5). Most active fractions are being analyzed by UPLC-HRMS using a high-throughput dereplication and molecular networking method (Figures 6, 7, 8).

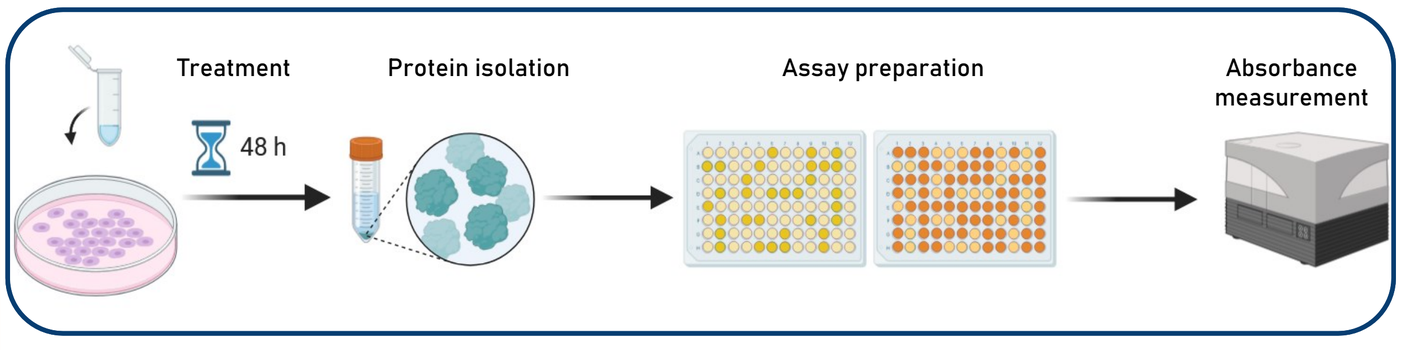


Figure 1. Methodology overview for cell-based tyrosinase and elastase inhibition assays. Cells (B16F10 and BJ) were treated with extracts (2 μg/ml) and incubated for 48h in 37°C, 5% CO_2 and 95% humidity. Cells were lysed with NP-40 lysis buffer and isolated proteins were plated in a 96-well plate with L-DOPA and elastase substrate respectively for tyrosinase and elastase. Absorbance was measured at 475 nm for tyrosinase and 405 nm for elastase assay.

Results

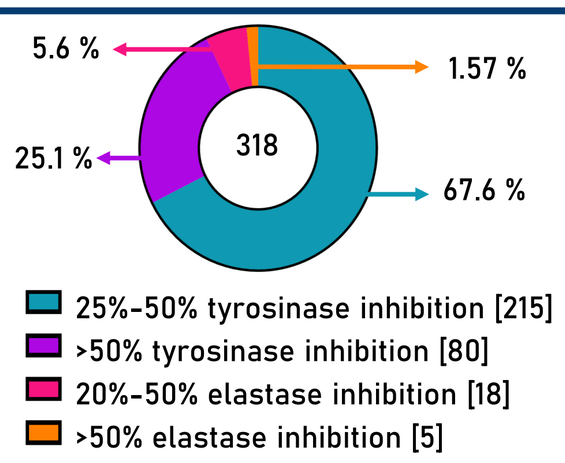


Figure 2. Enzymatic assays reveal 318 active extracts. Most of them exhibit tyrosinase inhibition (295 extracts) and fewer elastase inhibition (23 extracts).

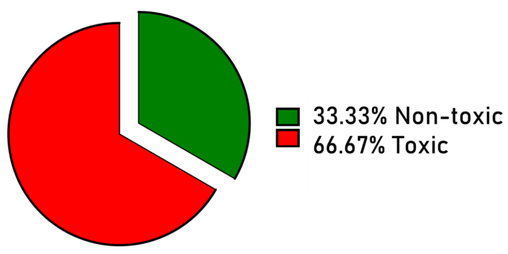


Figure 3. Out of 318, 93 extracts were tested for possible toxic activity. 33.3% of them were non-toxic in either BJ or HaCaT cells.

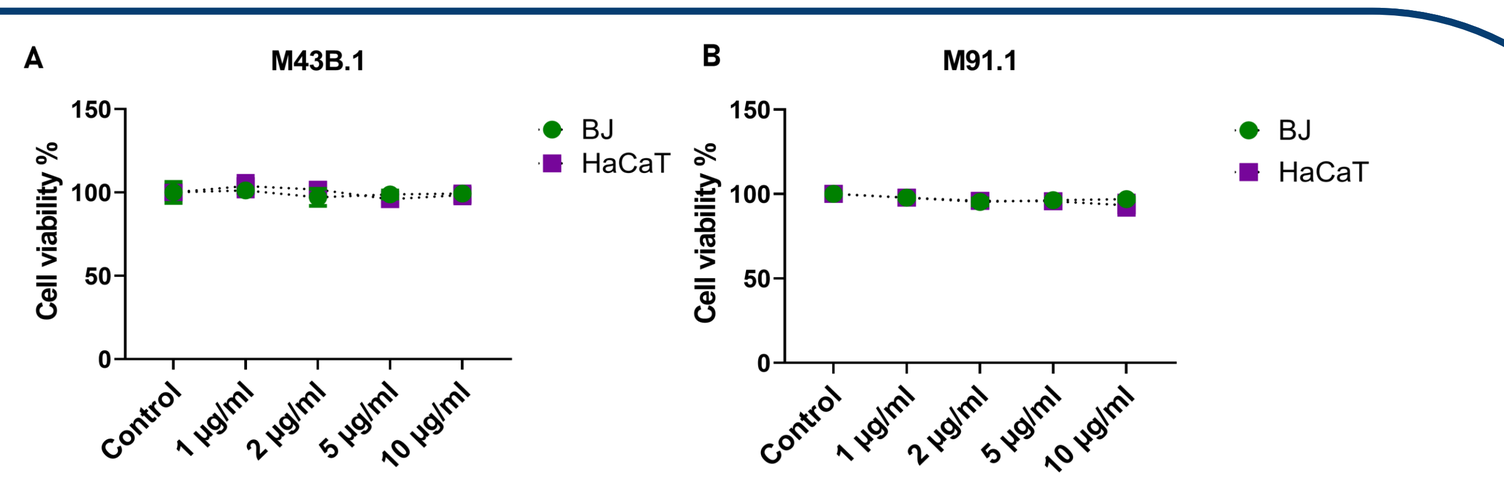


Figure 4. Extracts M43B.1 (A) and M91.1(B) appear to be non-toxic (>90% viability) in both BJ and HaCaT cells.

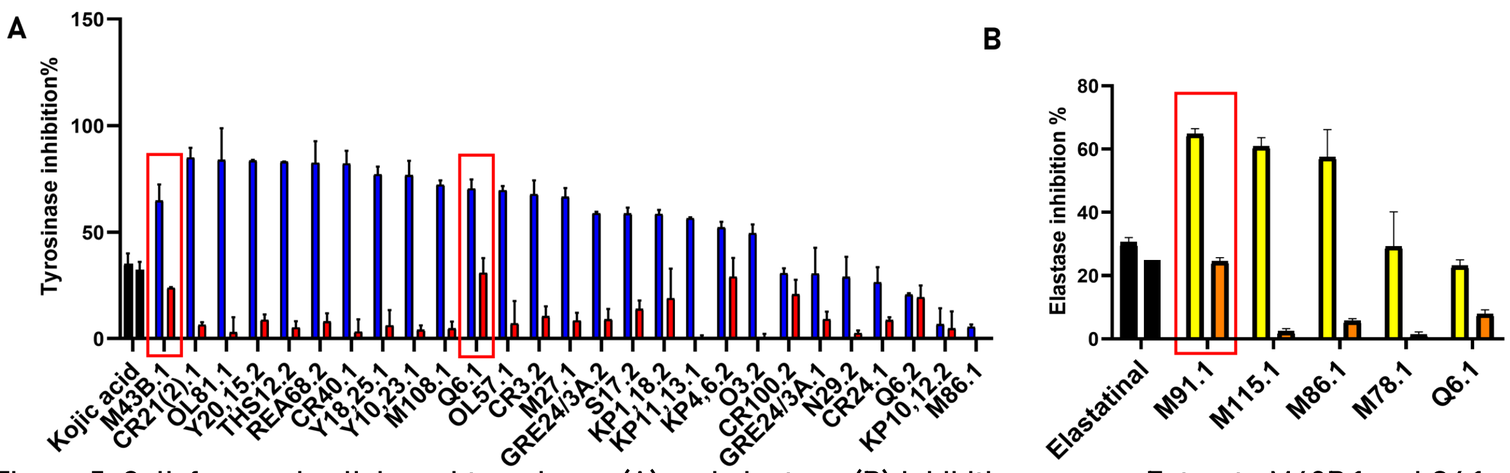


Figure 5. Cell-free and cell-based tyrosinase (A) and elastase (B) inhibition assays. Extracts M43B.1 and Q6.1 exhibit the highest anti-tyrosinase activity, while M91.1 the highest anti-elastase activity. Kojic acid (2 μg/ml, 100 μg/ml) and elastatinal (0,5 μg/ml) were used as positive controls. Blue,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

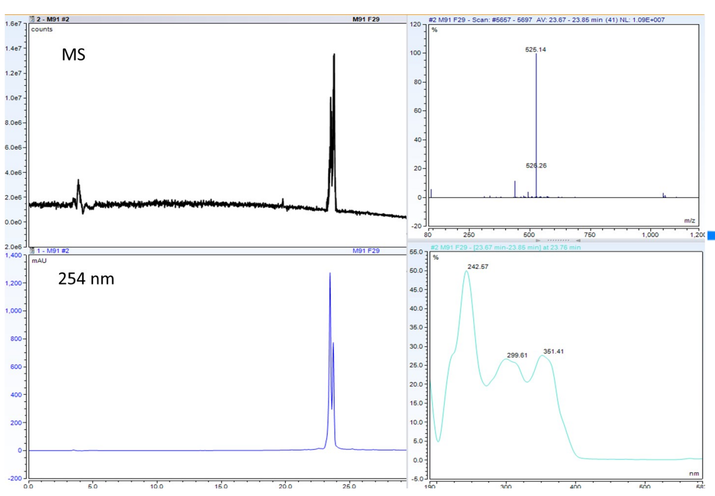


Figure 6. Chromatographic & spectroscopic data from Fraction 29 of M91 extract (Thermo Scientific™ ISQ™ EC Single Quadrupole Mass Spectrometer).

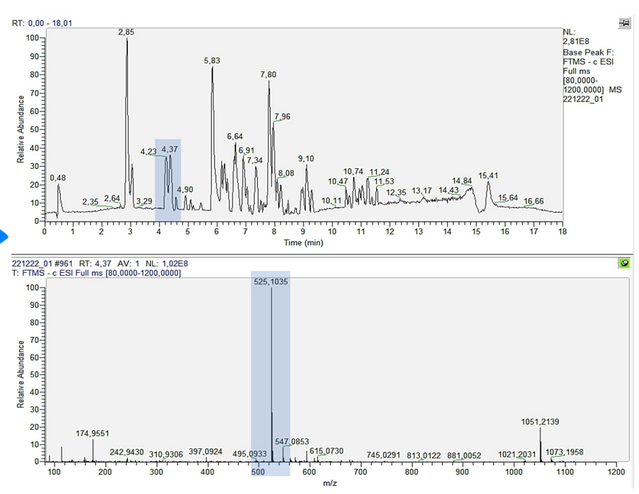


Figure 7. UHPLC-HRMS analysis of the M91 EtOAc extract (Thermo Scientific™ LTQ XL™ linear ion trap mass spectrometer).

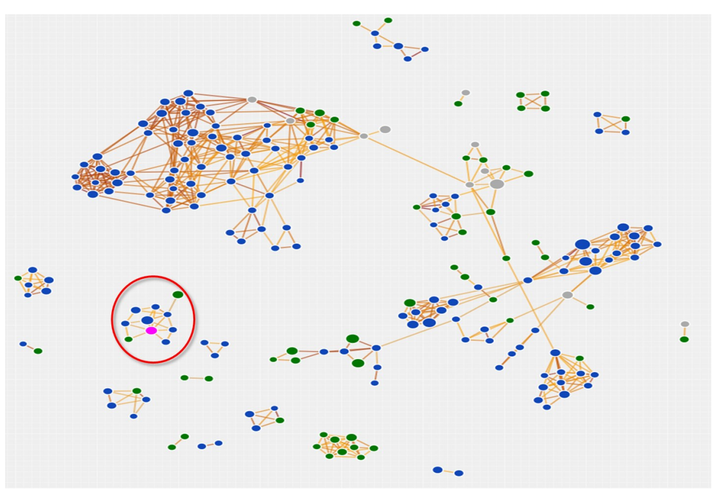


Figure 8. Molecular Network of M91 extract.
Legend: ● MS2 match; ● DDA for another ion; ● No MS2; ● Cluster of interest.

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