"AntiAging" Project

Exploitation of Greek microbial diversity for the development of innovative cosmeceuticals and food supplements

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I. Overview

- Greek ecosystems represent an under-explored ground for the study of Actinobacteria;
- This project aims to uncover potentially novel natural actinobacterial compounds with anti-aging activity that can be formulated as cosmeceutical and nutraceutical products;
- 1000 isolates belonging to the ATHUBA Culture Collection were studied;
- An ultrasound-assisted extraction process was designed using solvents of increasing polarity to generate a customized in-house library of 2000 extracts;
- 2000 extracts were investigated for their potential anti-aging properties via enzymatic anti-elastase and anti-tyrosinase activities, whereas cell-based assays are ongoing;
- Based on this bioassay-guided approach, the metabolic screening and compound annotation of the most promising isolates is conducted via a high-throughput dereplication method;
- This leads to a targeted isolation and identification of compounds using state-of-the-art chromatographic and spectroscopic techniques.

II. Workflow

Isolation of Actinobacteria (Actinomycetota) from diverse Greek ecosystems

Microscale cultivation of unique isolates.

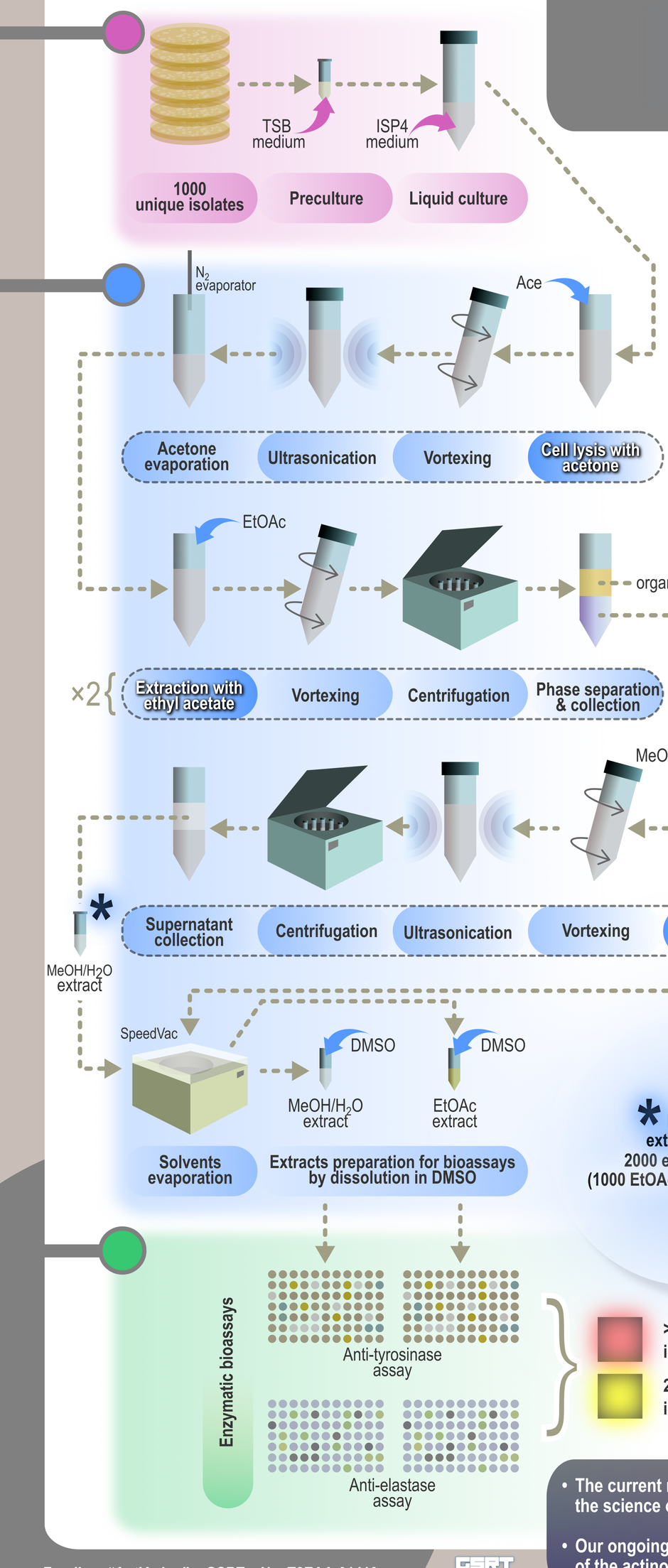
Development of an extraction protocol for the extraction of actinobacterial liquid cultures

Extraction of actinobacterial cultures **and creation of extract library**

Enzymatic and **cell-based bioassays**

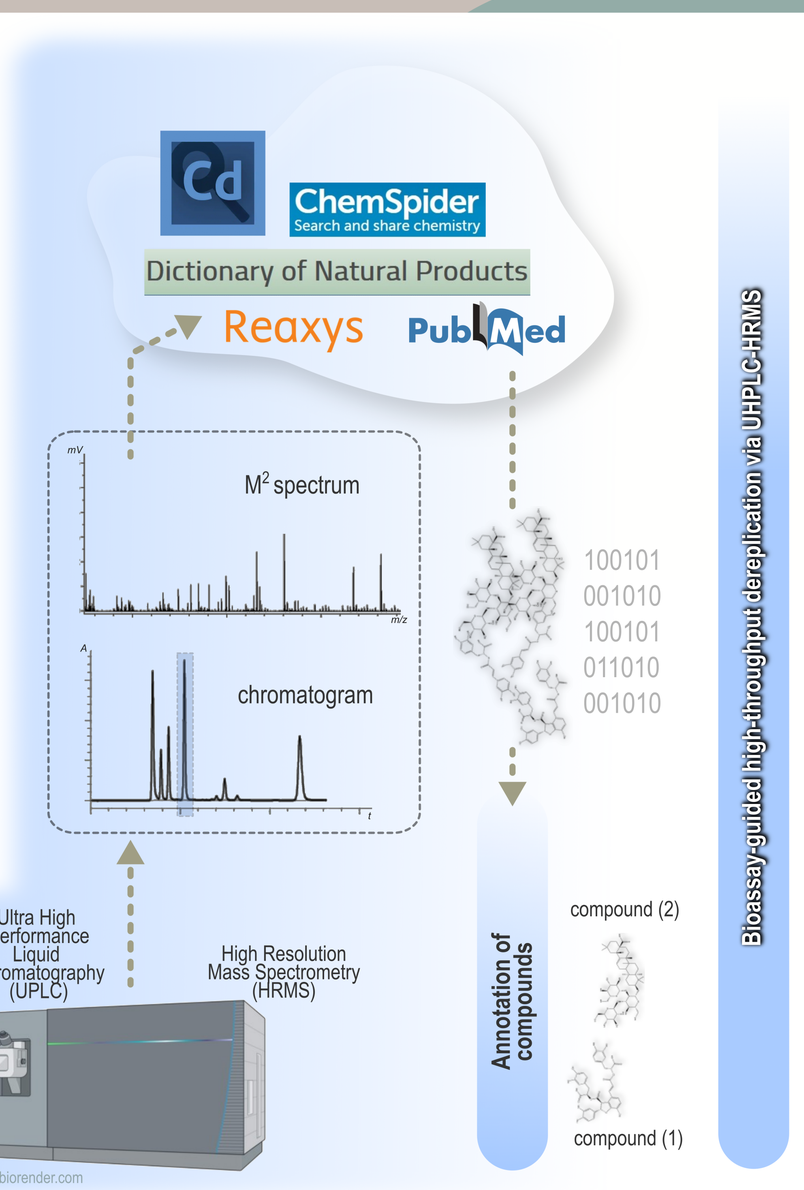
Bioassay-guided metabolic screening and compound annotation via **high-throughput dereplication method (UPLC-HRMS)**

III. Materials & Methods



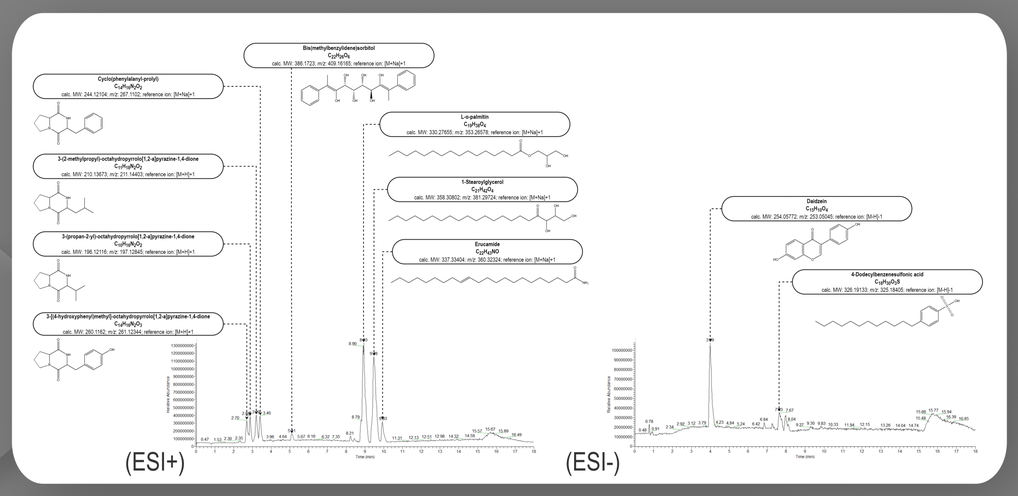
* In-house extract library: 2000 extracts in total (1000 EtOAc + 1000 MeOH/H_2O)

III. (cont.)

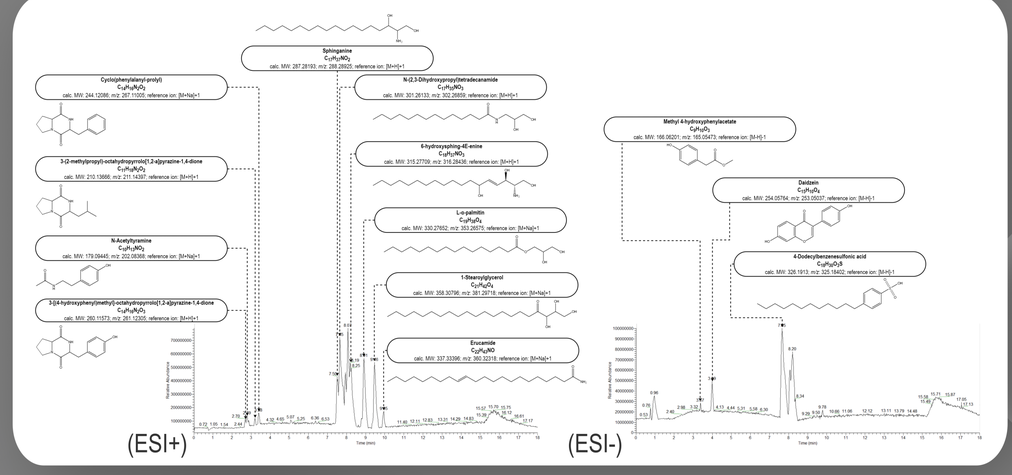


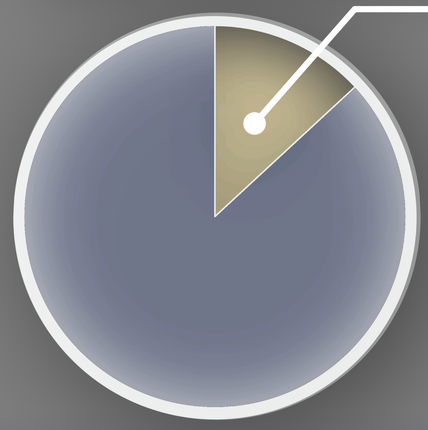
IV. Results & Progress

Ex. 1:
strain: **W8**
anti-TYR activity: **82.8%**



Ex. 2:
strain: **M91**
anti-ELA activity: **64.7%**





151 of 1000 isolates **[15.1%]** exhibit **anti-TYR** and/or **anti-ELA** bioactivity

of which

119 >50%
- **70** EtOAc (**5**: anti-ELA; **65** anti-TYR)
- **49** MeOH/H_2O (**all**: anti-TYR)

(>80%)
- (**16**: anti-TYR)
- (**5**: anti-TYR)

32 25-50%
- **14** EtOAc
- **18** MeOH/H_2O

V. Conclusions & Ongoing work

- The current results show a hidden yet powerful potential of harnessing Greek microbial wealth in the context of the science of anti-aging.
- Our ongoing work is focused on broadening the bioactivity tests from enzymatic to cell-based assays, as well as extending the dereplication methodology to the rest of the actinobacterial isolates, in order to determine the most promising ones for the scaled-up production and isolation of secondary metabolites.

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