

HELLENIC REPUBLIC
National and Kapodistrian University of Athens

Discovery of antiaging compounds via the screening of Greek actinobacteria

Laskaris Paris, Kaili Stavroula, Ntroumpogianni Georgia, Papageorgopoulos Ioannis, Katsifas Efstathios, Gaitanis Konstantinos, Tsafantakis Nikolaos, Sklirou Aimilia, Gkogkou Eirini, Hatzinikolaou Dimitris G., Fokialakis Nikolas, Trougakos Ioannis, Karagouni Amalia D.

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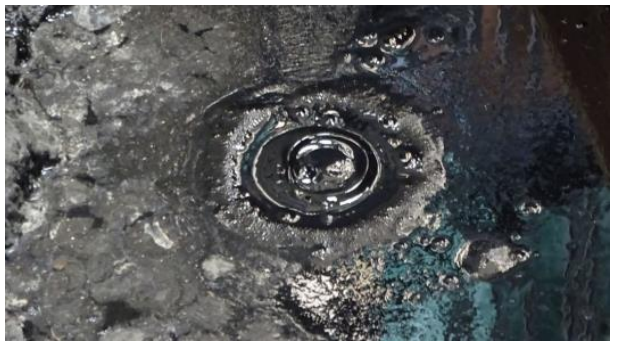
Greek microbial biodiversity

- Greece has a rich microbial diversity that produces a large number of secondary metabolites.

- Locations of research interest include:
  - Rhizosphere soils
  - Hot springs
  - Deep seas
  - Volcanic environments
  - Caves
  - Salt lakes
  - Hydrocarbon-contaminated regions



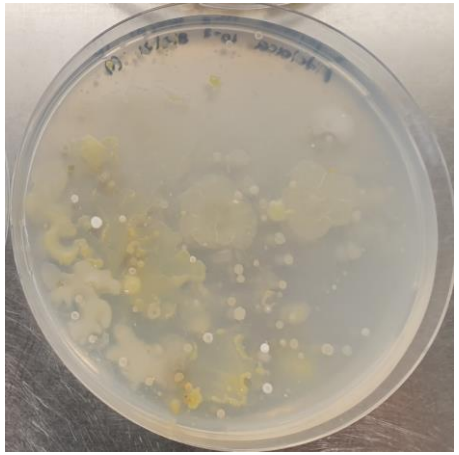




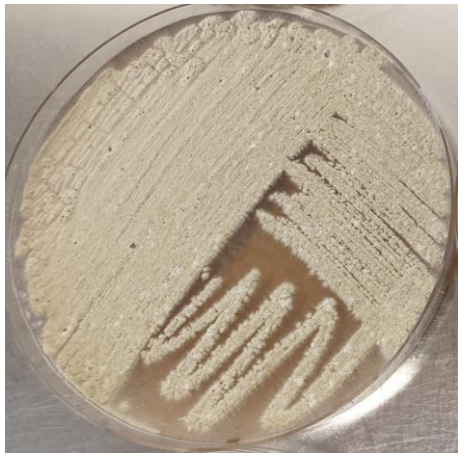
Athens University Bacterial & Archaeal Culture Collection (ATHUBA)



- The culture collection includes thousands of strains of the genus *Streptomyces* and other bacterial species isolated from a variety of Greek habitats.

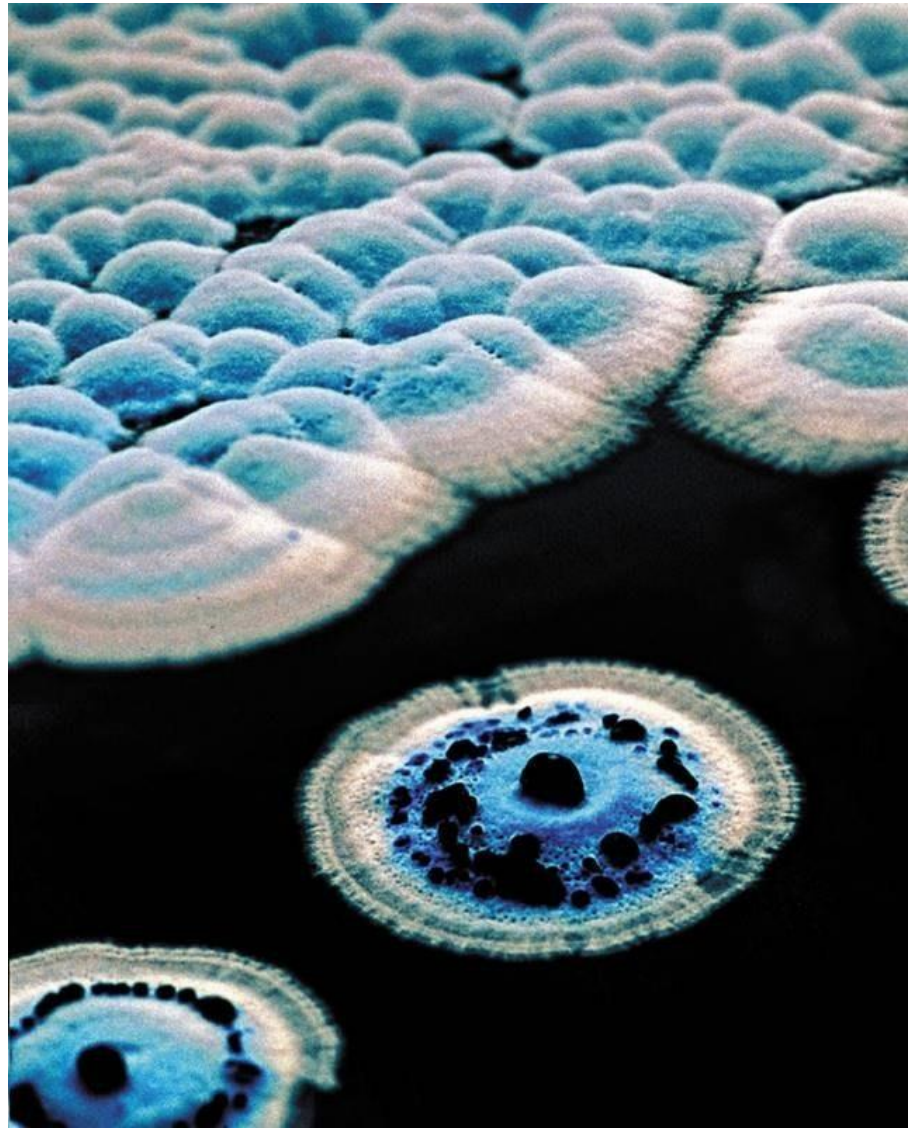


- Samples were collected from:
  - Soil microenvironments
  - Rhizospheres of endemic plants
  - Marine sediments
  - Hot springs



Biology of Genus *Streptomyces*

- *Streptomyces* genus belongs to phylum Actinomycetota.
- Procaryotic filamentous bacteria.
- Abundant in soil.
- Play a key role in recycling fungal and plant polymers.
- Important in specific environments such as oceanic sediments and in symbiosis with plants, fungi and animals.



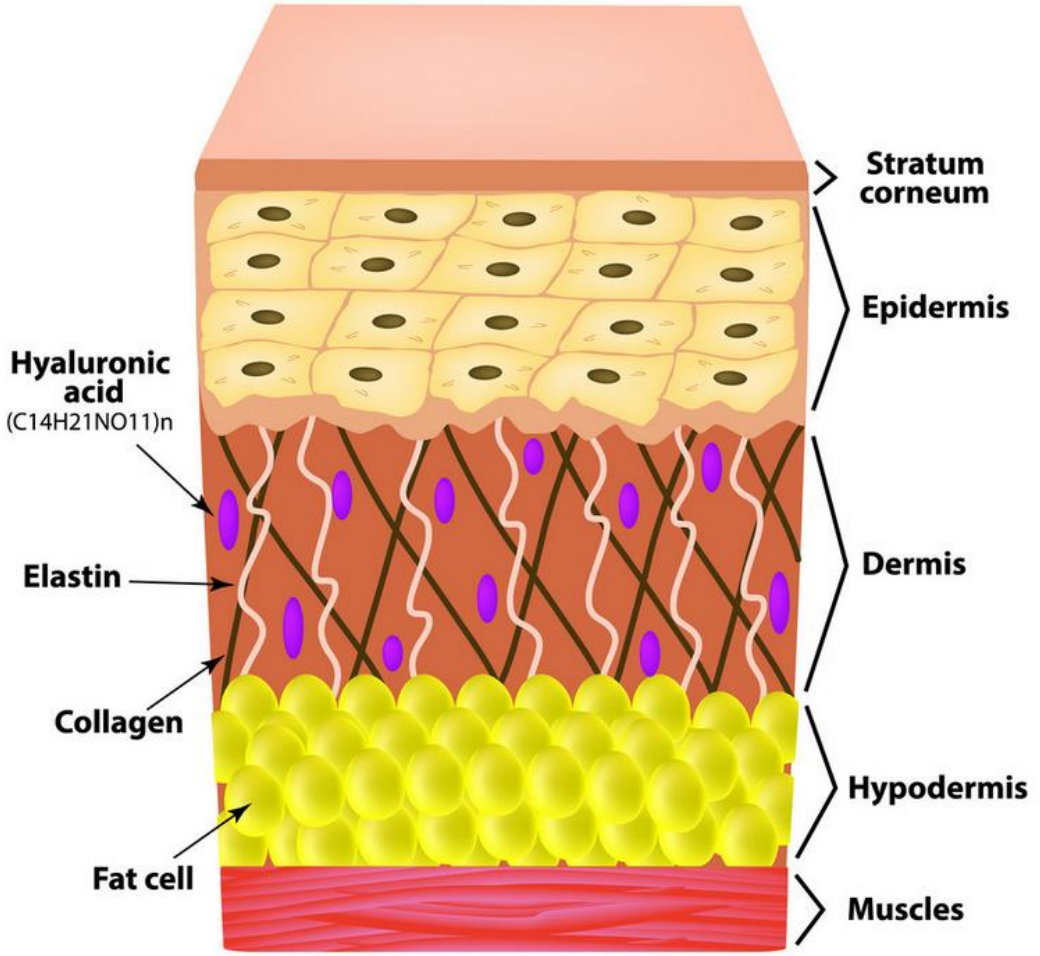
Streptomyces secondary metabolism

- *Streptomyces* strains produce:
  - 45% of known microbial metabolites
  - 17% all known active secondary metabolites
- Compounds they produce play in role in:
  - Medicine (wide variety of drugs)
  - Agriculture (plant growth promoters and natural pesticides)
  - Environmental stewardship (bioremediation)
  - Shipping (antifouling)



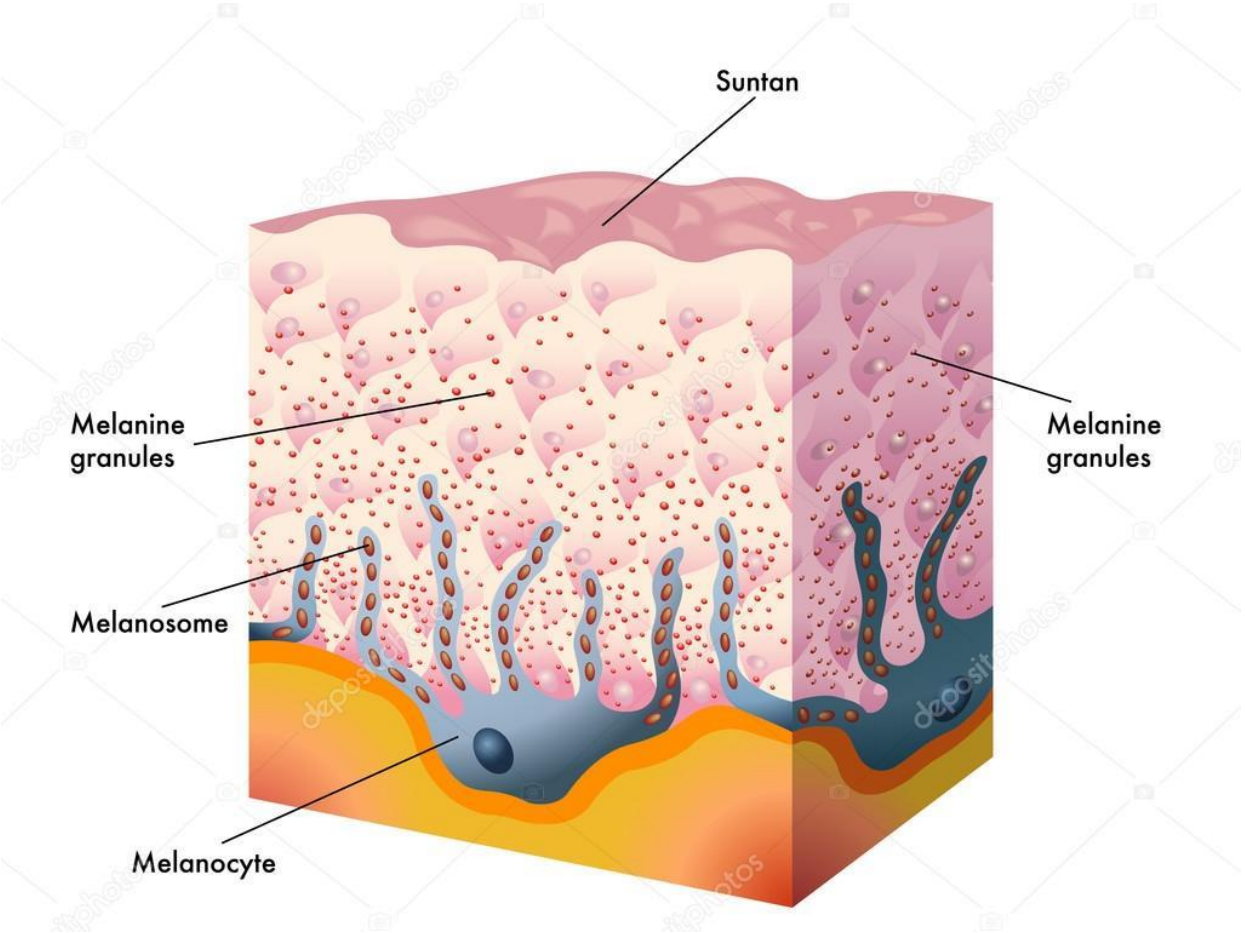
Anti-wrinkling compounds

- Elastin is vital for the elasticity of the skin and aging causes its levels to decrease.
- Elastase breaks down elastin.
- Molecules that inhibit the action of elastase could slow skin aging.



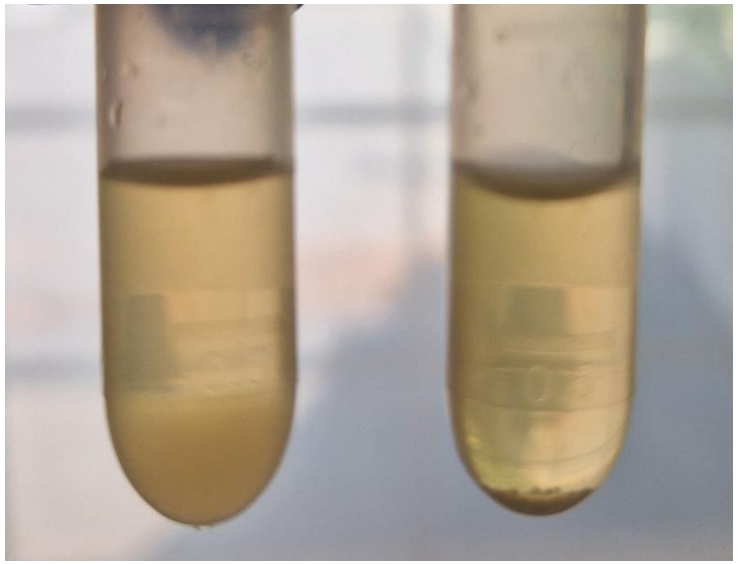
Skin whitening compounds

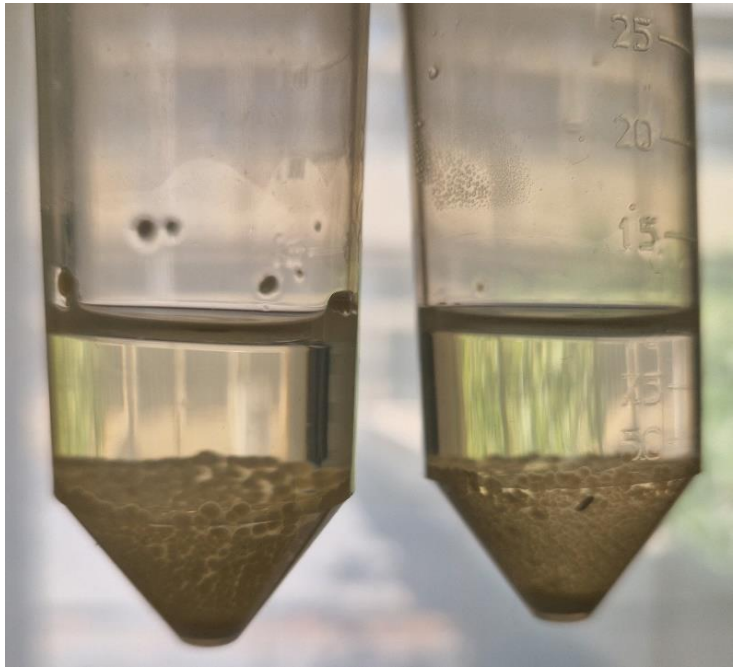
- Overproduction of melanin can result in age spots.
- Melanin synthesis requires the enzyme tyrosinase.
- Tyrosinase inhibitors can be used for skin whitening.



Streptomyces screen

- 2000 extracts of 1000 isolates were screened for inhibitory activity against the enzymes:
  - elastase (anti-wrinkle)
  - tyrosinase (skin whitening activity)





- Strains were precultured for 3 days in TSB (Tryptone Soy Broth) followed by culturing for 7 days in ISP4 (Inorganic Salt Starch Broth).

Extraction protocol

- **Step I**

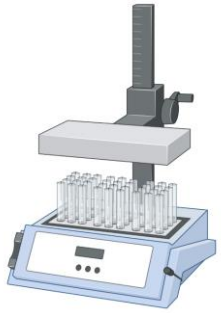




1 min vortexing



45 min UAE



Evaporation of acetone (N_2)

Cell lysis

- **Step II**





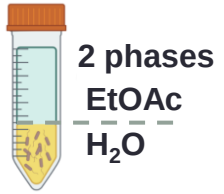
1 min vortexing



45 min UAE



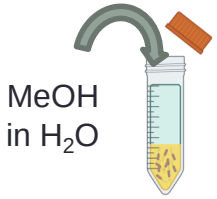
15 min centrifugation



Upper phase removal



evaporation





1 min vortexing



45 min UAE



15 min centrifugation



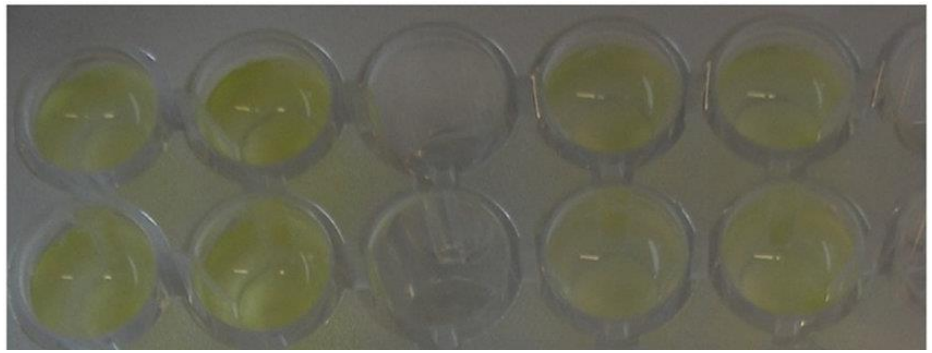
1,5 mL MeOH



evaporation

Elastase and tyrosinase inhibition assays

- Enzyme activity of elastase from porcine pancreas.
- N-succinyl-Ala-Ala-Ala-p-nitroanilide used as substrate.
- Released p-nitroaniline was measured spectrophotometrically.



- Enzyme activity of mushroom tyrosinase.
- L-DOPA used as substrate.
- Dopachrome formation was measured spectrophotometrically.

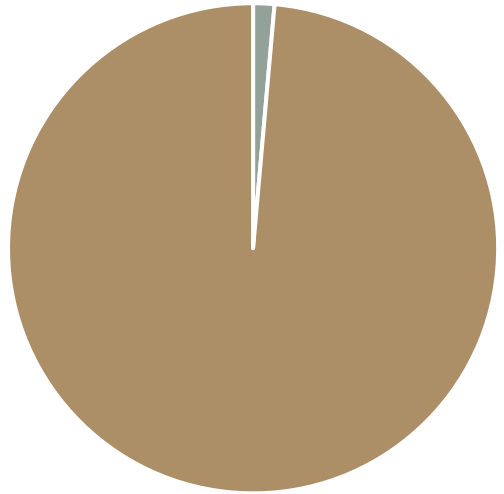
Enzymatic assay results

Number of positive results

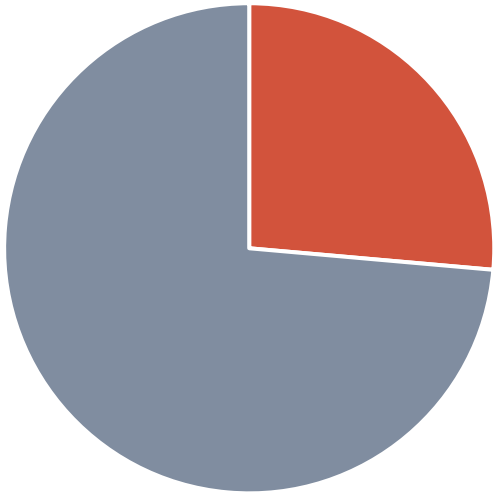
Solvent	Inhibition	Elastase	Tyrosinase
Ethyl acetate	>50%	5	57
Ethyl acetate	25% - 50%	9	125
Methanol	>50%	0	30
Methanol	25% - 50%	0	113

Enzymatic assay results

- 1.4% of strains had extracts that demonstrated elastase inhibition (14 total).
- 26.4% of strains had extracts that demonstrated tyrosinase inhibition (264 total).
- Compounds that inhibit tyrosinase are more common.
- Ethyl acetate proved a more effective solvent than methanol.



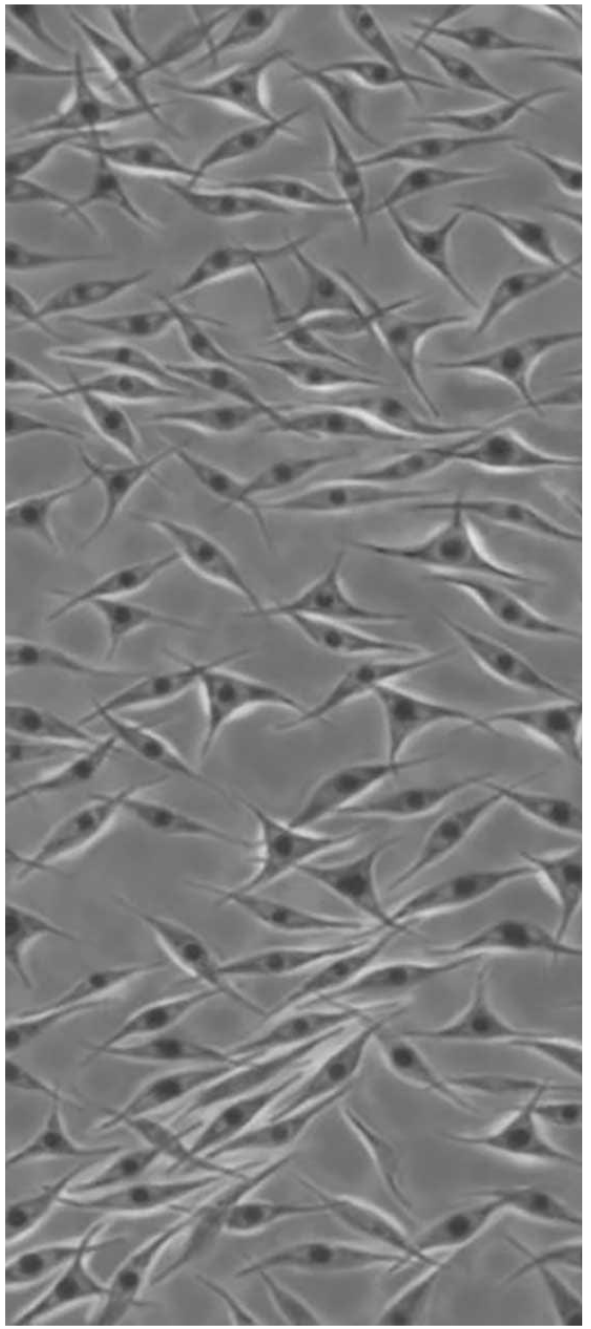
Elastase inhibition



Tyrosinase inhibition

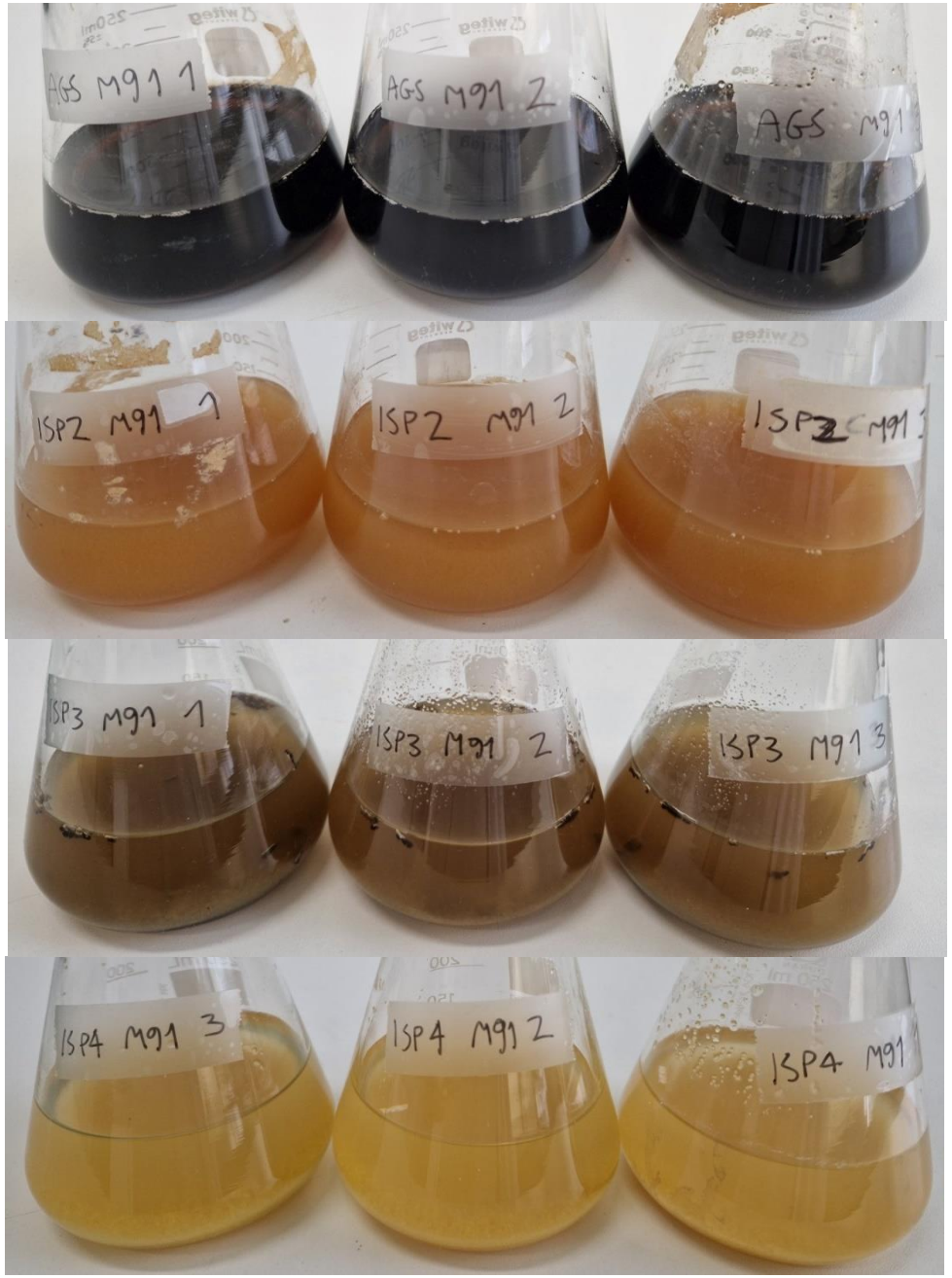
In vivo enzymatic inhibition

- The 100 most active extracts were tested for cytotoxicity on the BJ human fibloblast cell line and the HaCaT human keratinocyte cell line.
- 37 extracts (37%) were found to be non-cytotoxic.
- Tested for tyrosinase inhibition on the B16F10 murine melanoma cell line.
- Tested for elastase inhibition on the BJ human fibloblast cell line.
- 3 extracts inhibit tyrosinase *in vivo* (13% of non-cytotoxic extracts).
- 1 extract inhibits elastase *in vivo* (25%).



Metabolite production optimization

- Strains producing extracts active *in vivo* were tested on 4 different media (AGS, ISP2, ISP3, ISP4) to attempt and increase yields.
- The extracts from these cultures underwent *in vitro* inhibition assays on elastase or tyrosinase and their effectiveness was compared.
- It was determined that ISP4 was the most optimal medium for 3 strains.
- 1 tyrosinase inhibiting strain failed to show activity in these assays.

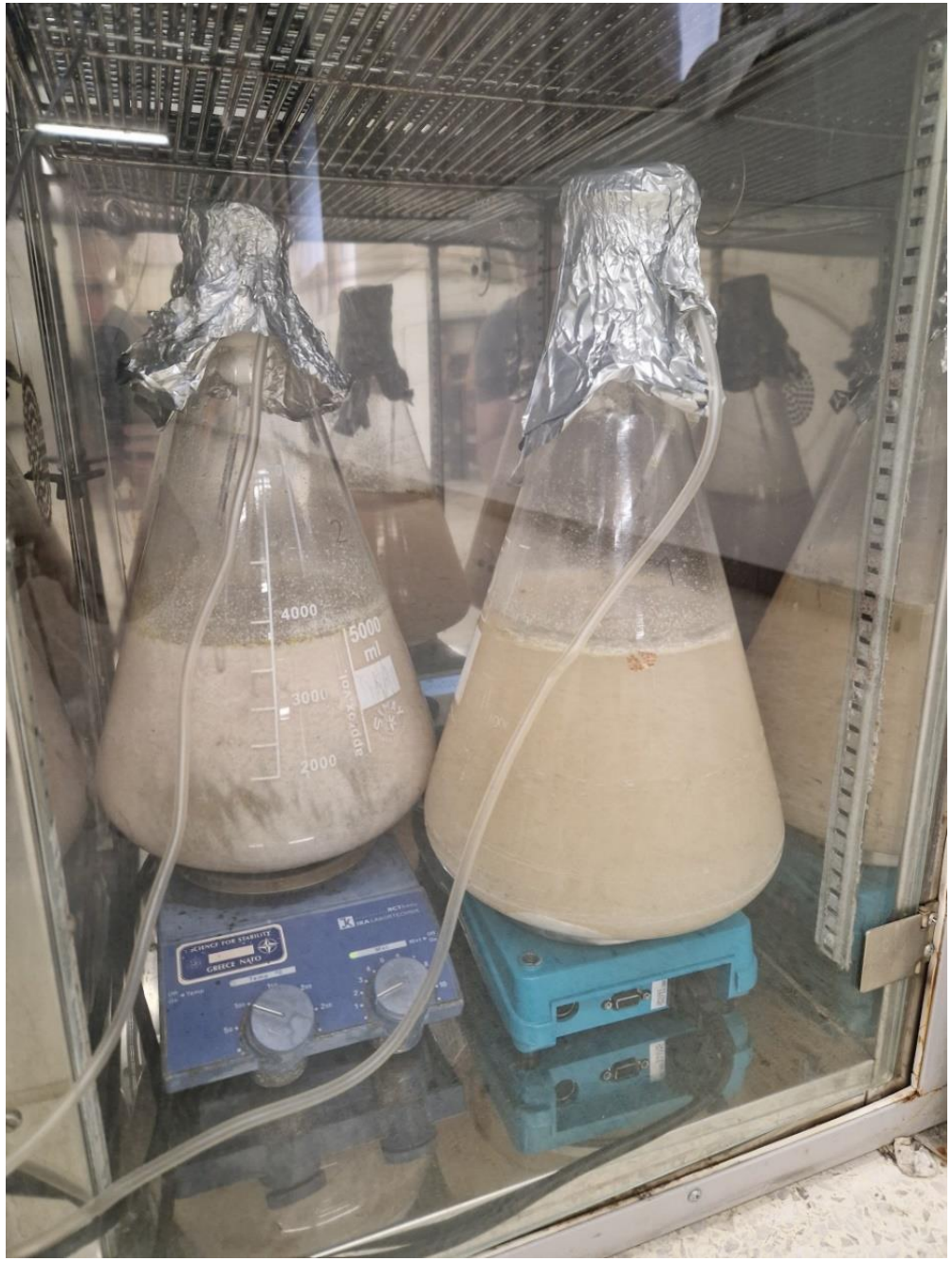


Conclusions

- ISP4 (Inorganic Salt Starch Broth) proved to be the optimal medium both for the 1000 strain screening and for large scale culturing.
- Ethyl acetate was a more effective solvent than methanol.
- Tyrosinase inhibition (26.4%) was considerably more widespread than elastase inhibition (1.4%).
- 37% of all tested extracts were non-cytotoxic.
- 13% of non-cytotoxic extracts that inhibited tyrosinase *in vitro* also did so *in vivo.*
- 25% of non-cytotoxic extracts that inhibited elastase *in vitro* also did so *in vivo.*

Future work

- The 3 most promising strains were cultivated in a larger scale and extracts made.
- Extracts are undergoing fractionation and the fractions subjected to *in vitro* inhibition assays to identify the active compounds.
- A commercial partner can develop products that incorporate these compounds.









Acknowledgements

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ΕΥΔΕ ΕΤΑΚ

Ερευνώ, Δημιουργώ, Καινοτομώ