

HELLENIC REPUBLIC
National and Kapodistrian University of Athens

Screening a Greek actinobacterial culture collection for antiaging compound producers

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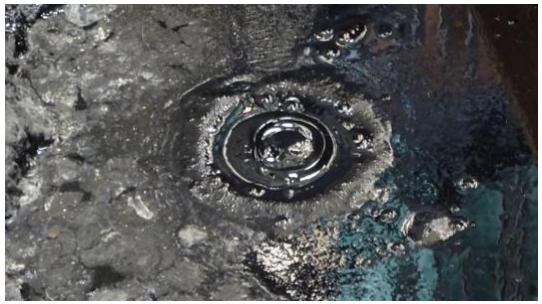
ECCO XL, Braunschweig, 29 September 2022

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





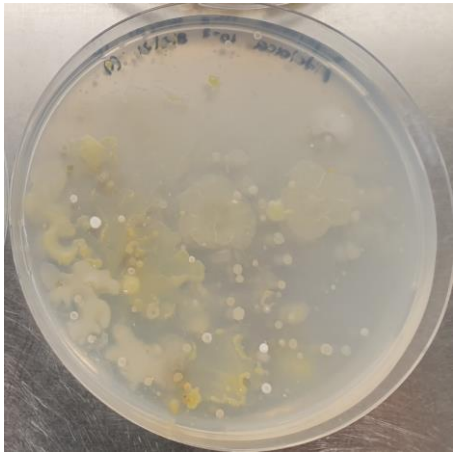


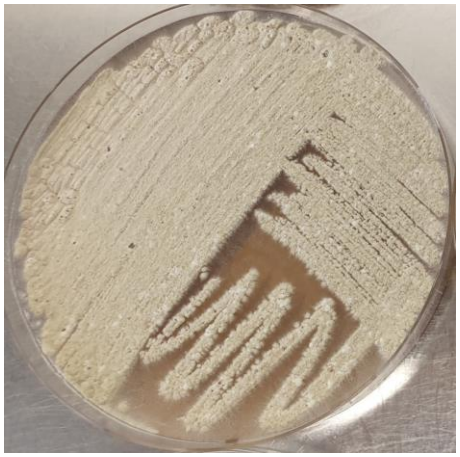
Athens University Bacterial & Archaeal Culture Collection (ATHUBA)

- 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

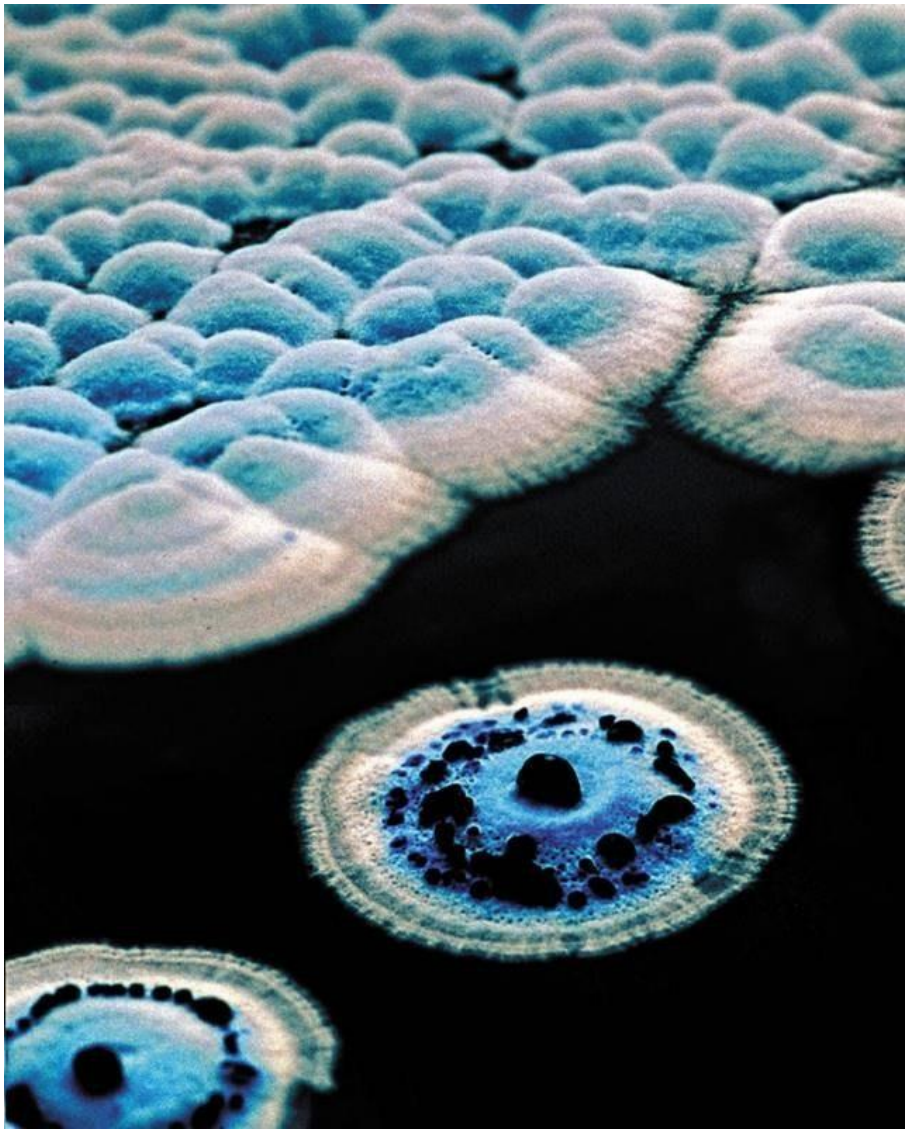




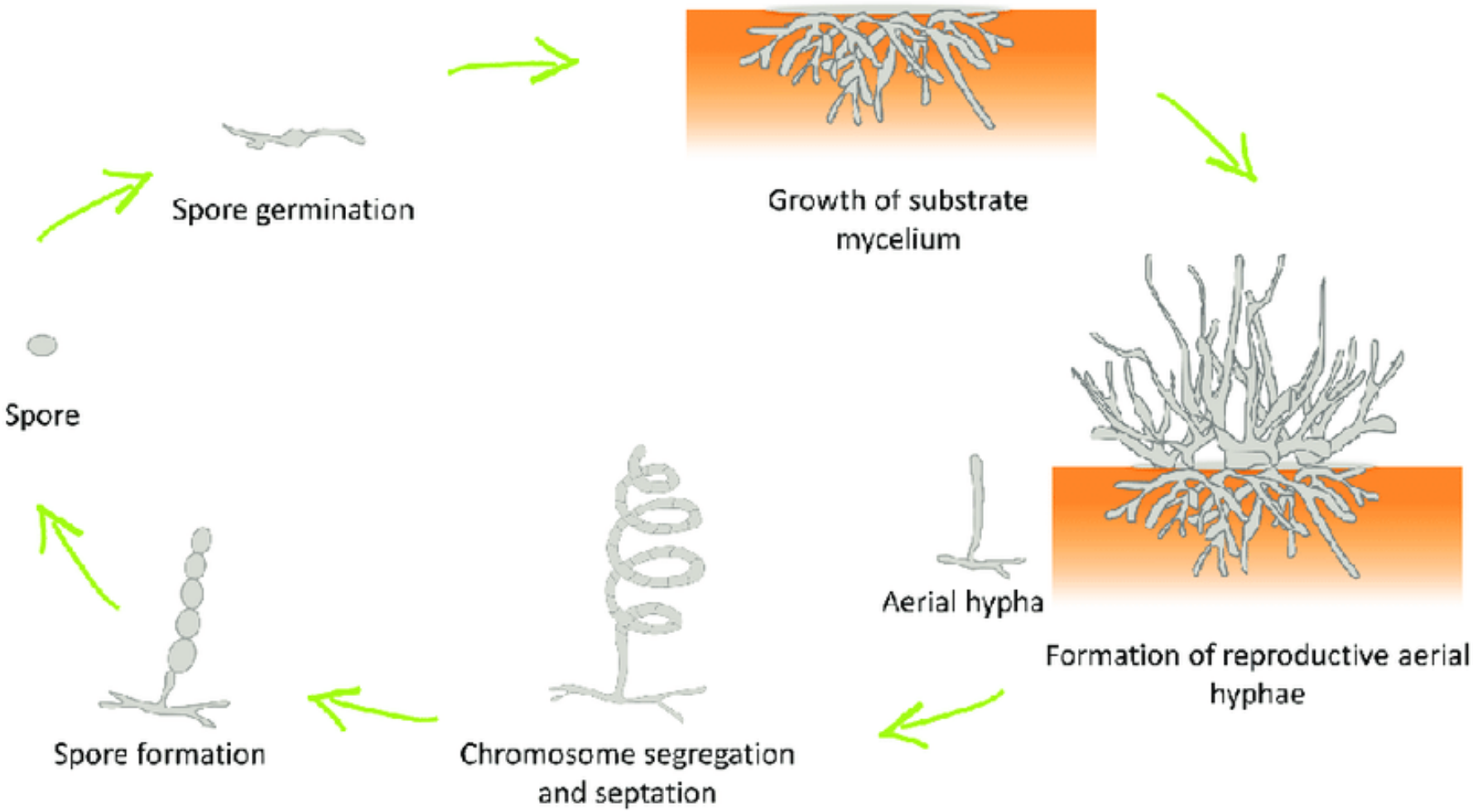


Genus *Streptomyces*

- *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.

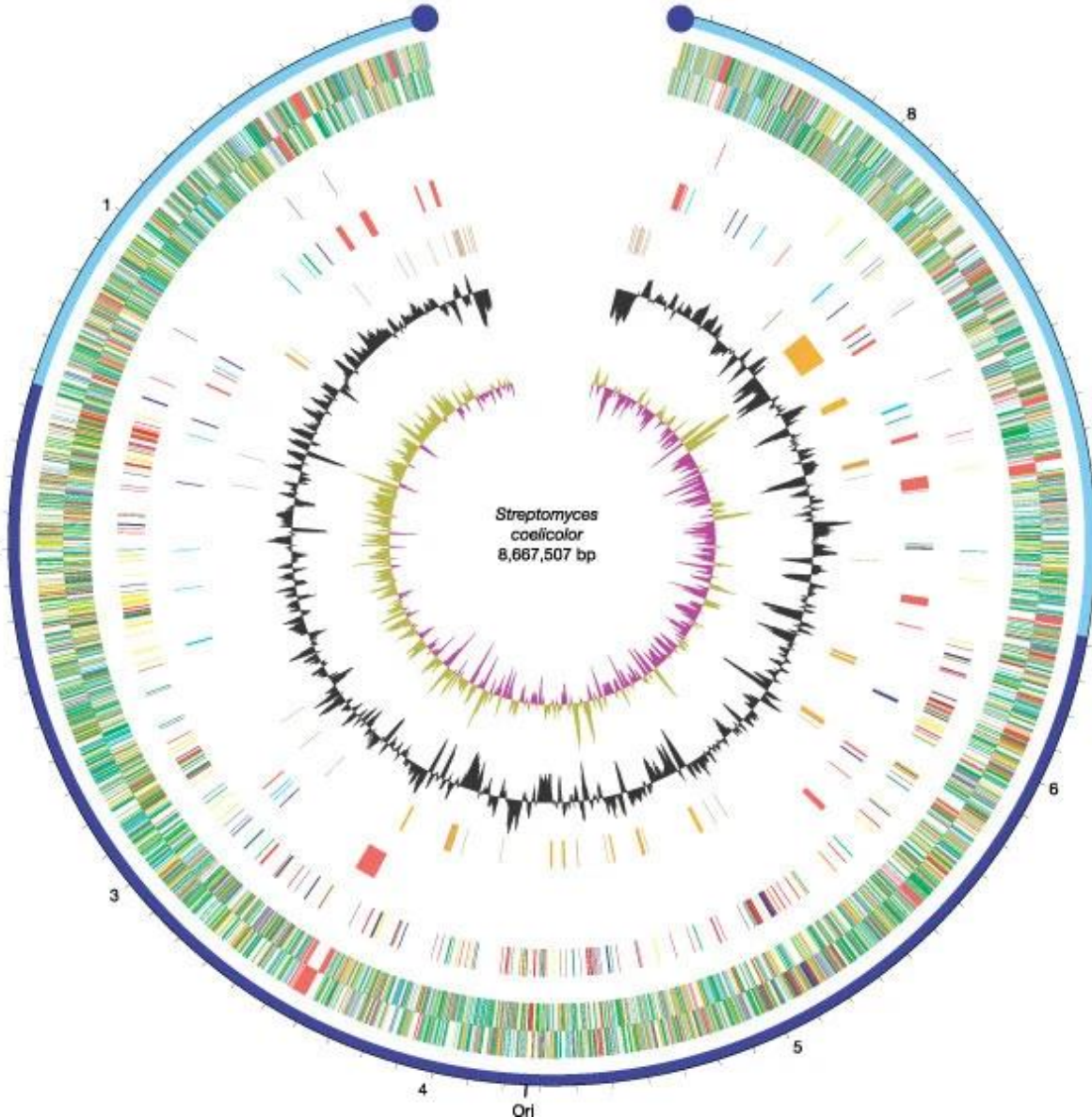


Lifecycle of *Streptomyces*



Streptomyces genome

- *Streptomyces* have a linear chromosome that can range from 6 Mb to 12 Mb.
- Have an average of 7000 protein coding genes.
- On average can potentially produce more than 30 secondary metabolites.



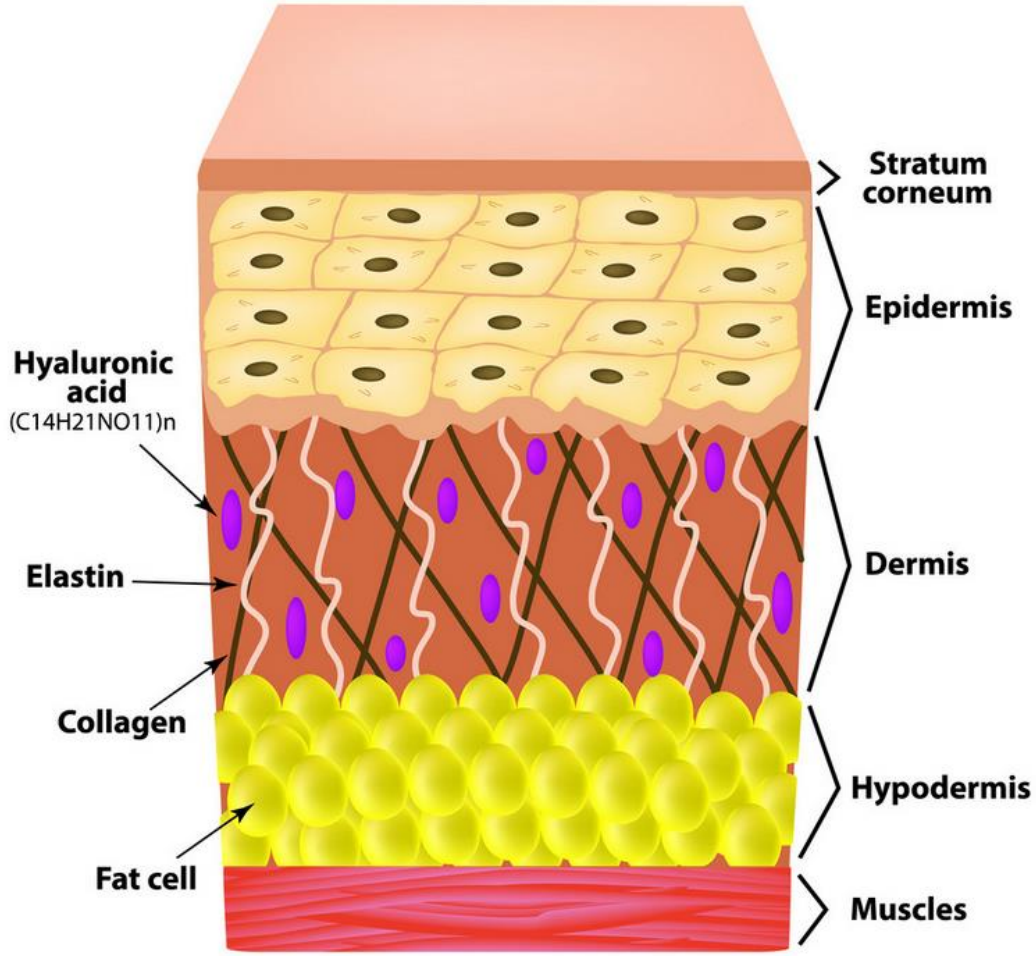
Streptomyces secondary metabolism

- *Streptomyces* strains produce:
  - 45% of 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)



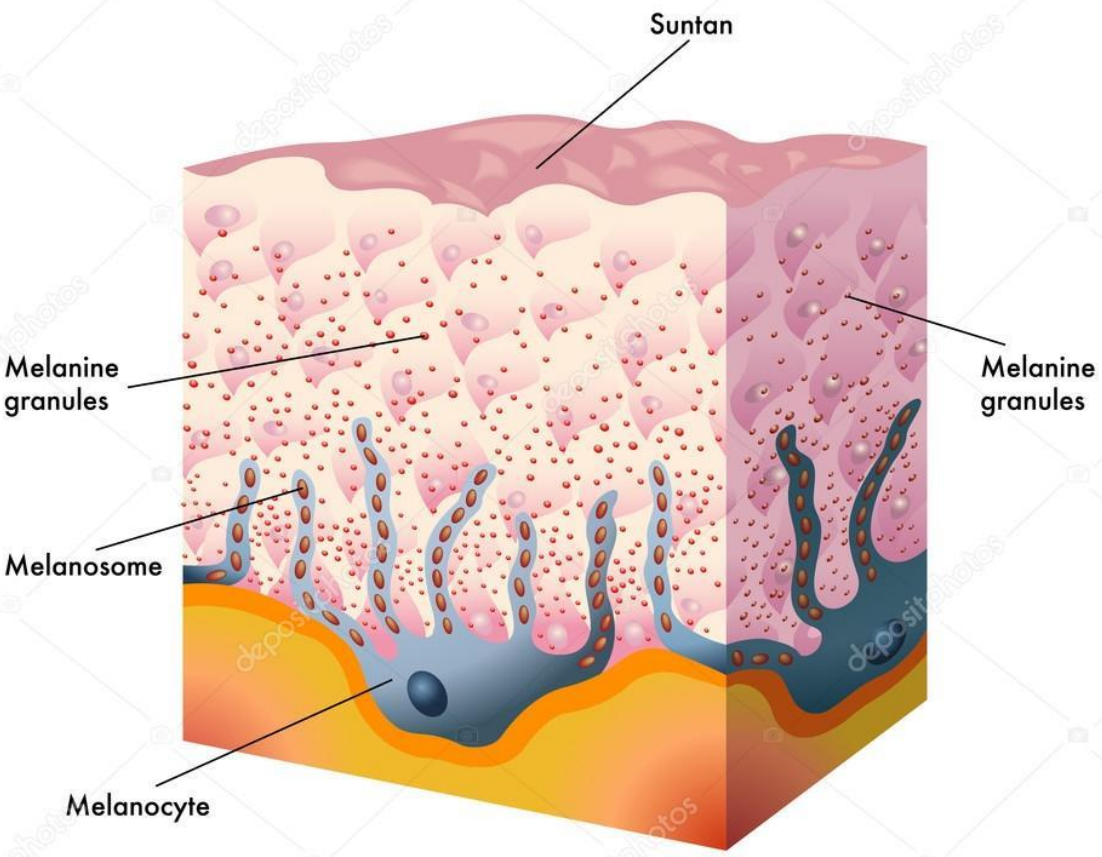
Anti-wrinkling compounds

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



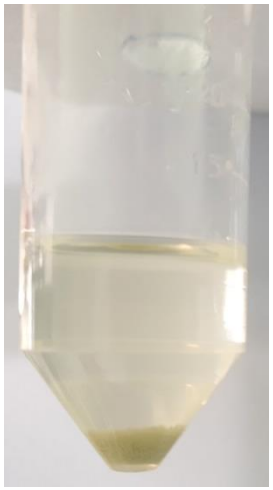
Skin whitening compounds

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



Streptomyces media selection

- Media widely used for the production of *Streptomyces* secondary metabolites in liquid culture:
  - AGS (Arginine Glycerol Salt)
  - ISP1 (Tryptone Yeast Extract)
  - ISP2 (Yeast Malt)
  - ISP4 (Inorganic Salt Starch)
  - M1 (Starch Yeast Peptone)
- ISP4 selected:
  - its components were not extracted
  - had satisfactory biomass production



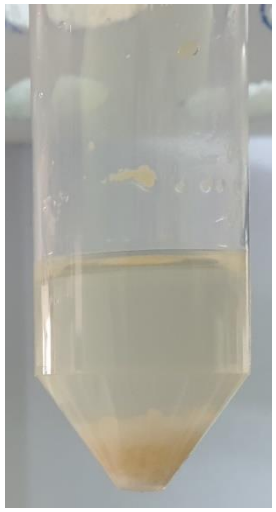
AGS



ISP1



ISP2



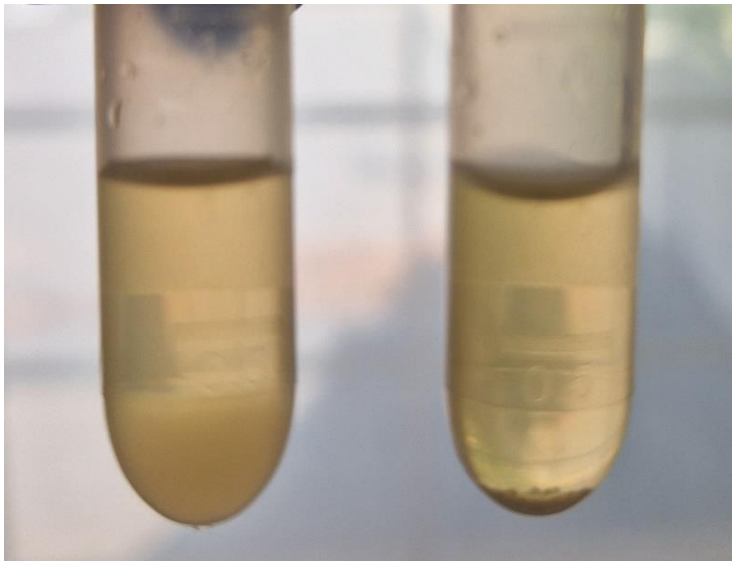
ISP4

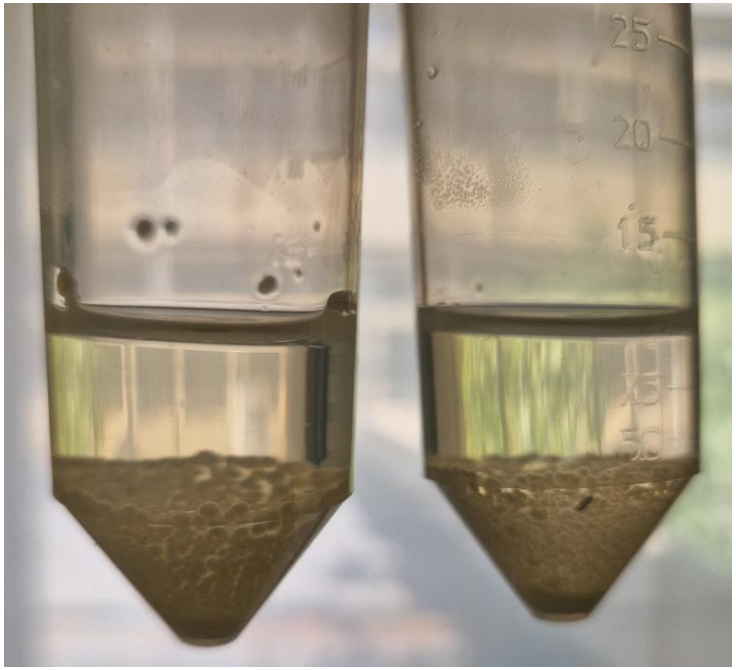


M1

Streptomyces screen

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

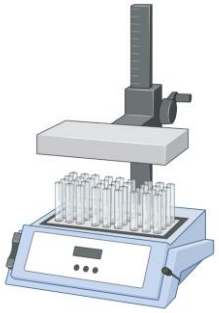




1 min vortexing



45 min UAE



Evaporation of acetone (N_2)

Cell lysis

- **Step II**





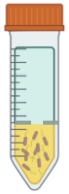
1 min vortexing



45 min UAE



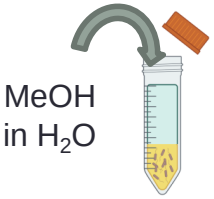
15 min centrifugation



Upper phase removal



evaporation





1 min vortexing



45 min UAE



15 min centrifugation



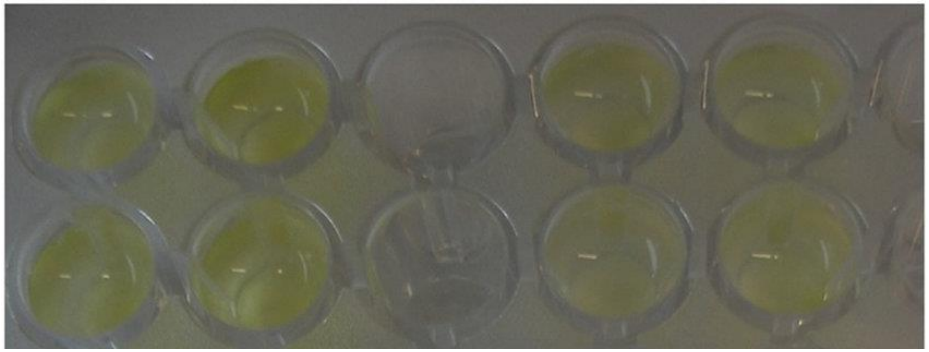
1,5 mL MeOH



evaporation

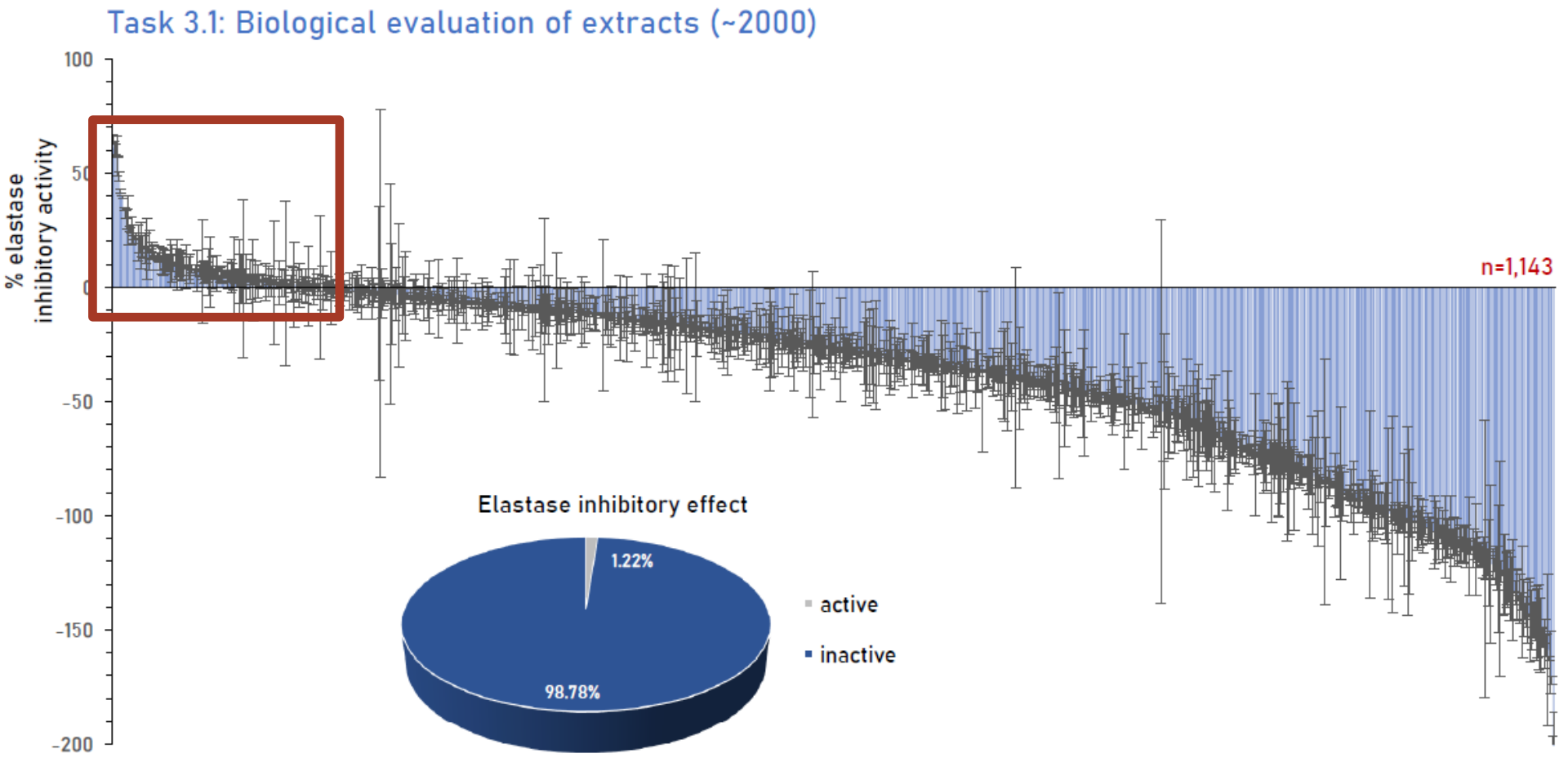
Elastase and tyrosinase inhibition assays

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

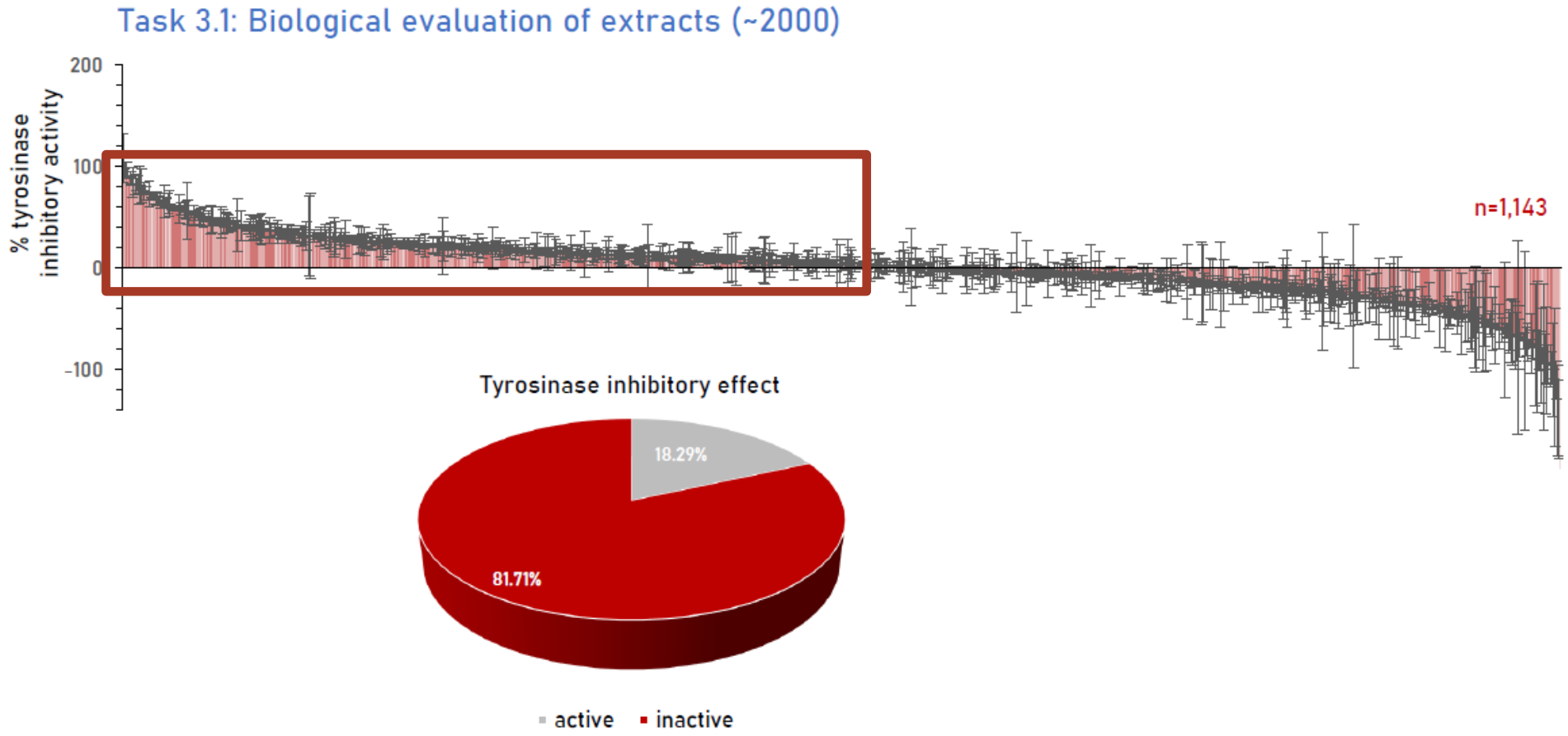


- Enzyme activity of mushroom tyrosinase.
- L-DOPA used as substrate.
- Dopachrome formation was measured spectrophotometrically.

Elastase inhibition screen results



Tyrosinase inhibition screen results



Assay results

Number of positive results

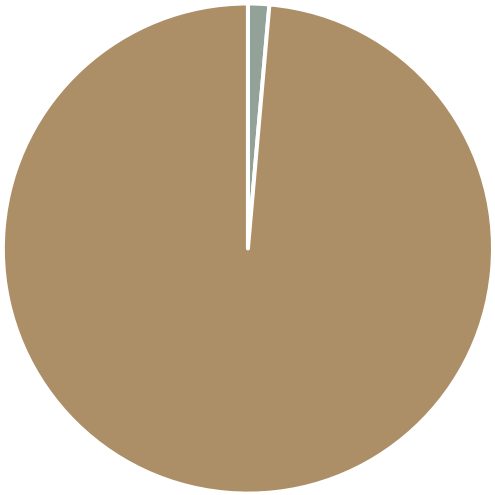
Solvent	Inhibition	Elastase	Tyrosinase
Ethyl acetate	>50%	5	57
Ethyl acetate	25% - 50%	9	125
Methanol	>50%	0	30
Methanol	25% - 50%	0	113

Percentage of positive results

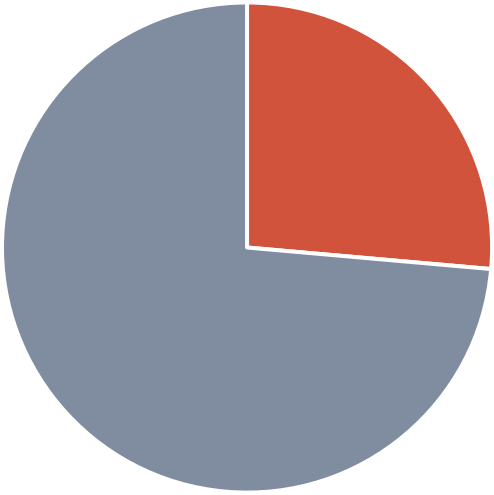
Solvent	Inhibition	Elastase	Tyrosinase
Ethyl acetate	>50%	0.5%	5.7%
Ethyl acetate	25% - 50%	0.9%	12.5%
Methanol	>50%	0%	3%
Methanol	25% - 50%	0%	11.3%

Conclusions

- 1.4% of strain extracts demonstrated elastase inhibition. (14 total)
- 26.4% of strain extracts demonstrated tyrosinase inhibition (264 total).
- Tyrosinase is more sensitive than elastase.
- Ethyl acetate proved a more effective solvent than methanol.



Elastase inhibition



Tyrosinase inhibition

Future work

- Cultivation of selected strains will be scaled up in bioreactors.
- Fractionation will be used to isolate active compounds.
- Compound effects will be tested on live cells to confirm activity.
- Tests will be conducted on human cell lines to ensure the compounds are non-toxic.
- A commercial partner will develop products that incorporate these compounds.









Acknowledgements

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