EXPLOITATION OF GLOBAL MICROBIAL BIODIVERSITY FOR THE DISCOVERY OF NOVEL COSMECEUTICALS USING LC-HRMS BASED METABOLOMICS

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MICROSMETICS — INNOVATIVE COSMETICS FROM MICROORGANISMS

Abstract

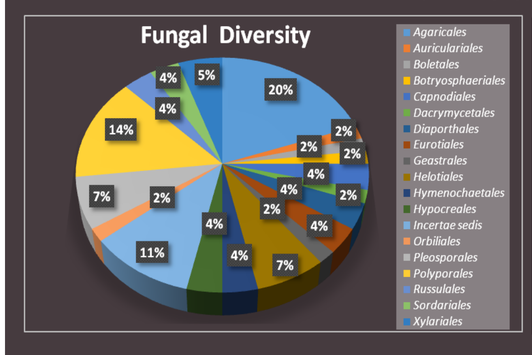
• MICROSMETICS, is an EU funded project aiming to discover and bring to development innovative anti-ageing cosmeceuticals, originating from microbial biodiversity, using emerging and state of the art technologies in the field of biotechnology, natural products chemistry and applied microbiology.

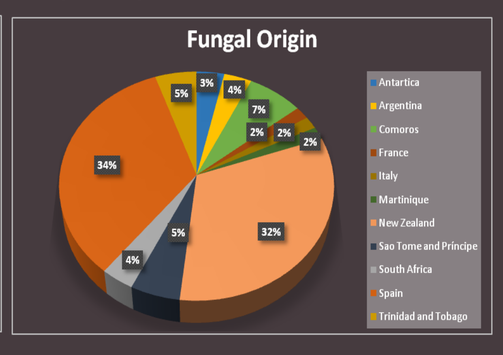
• The proprietary microbial collection of Fundación MEDINA (over 116.000 strains) is being exploited by incorporating modern high throughput screening platforms (*in silico* & *in vitro*) for the rational and targeted selection of the most promising strains. Advanced analytical approaches and techniques are being applied for the efficient, accelerated and advantageous isolation and identification of natural constituents. A broad spectrum of bioassays and metabolomics approaches are being incorporated for the evaluation of anti-ageing, more specifically anti-oxidant, skin-protecting, and skin-whitening activity of all derived molecules.

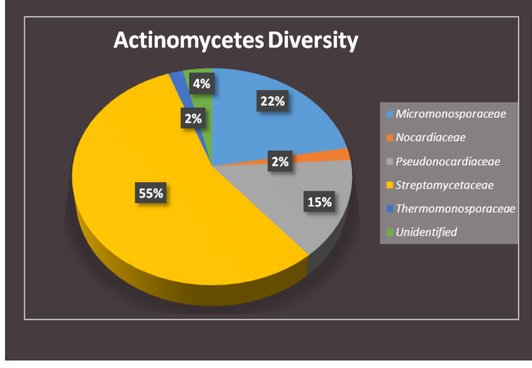
• In the frame of MICROSMETICS, 40.000 known microbial metabolites from the dictionary of microbial natural products ANTIBASE were screened in silico against selected biological targets with cosmeceutical interest (Tyrosinase, Elastase, Collagenase). More than 110 potential candidate strains identified from a Rational Drug Design Tool (using a functional prediction model, virtual screening and similarity search) were selected to be studied. Among them 55 fungi and 55 actinomycetes were cultivated under “nutritional arrays”. Approximately 1100 extracts have been generated and evaluated for their biological activity. Among them the top 100 were selected and a strategy combining UHPLC/Orbitrap-HRMS, in positive and negative modes, with multivariate statistical methods was applied. All derived chromatograms have been analyzed and a positive correlation between the profiles of the extracts with the aforementioned bioassays was observed. Thus, the 10 most promising extracts that represent the clusters generated in metabolomics have been selected for large-scale cultivation and bioassay guided isolation of potential novel molecules.

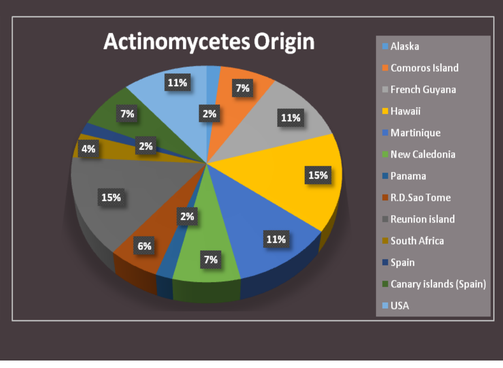
EXPERIMENTAL

DIVERSITY OF STRAINS SELECTED FROM RATIONAL BIOPROSPECTING





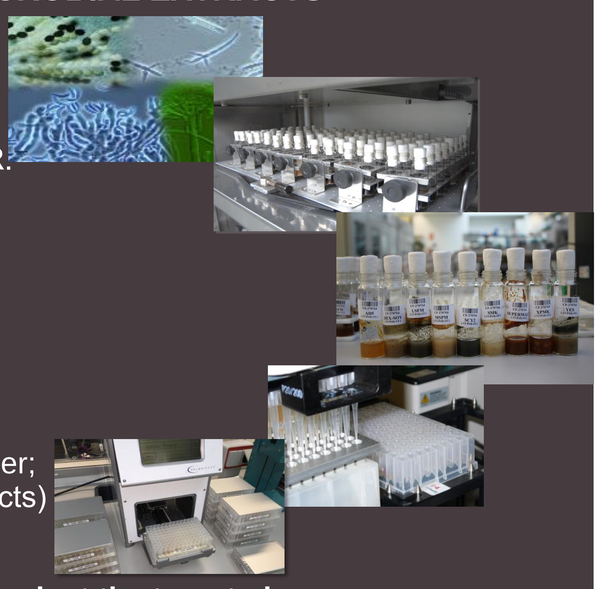




HIGH THROUGHPUT PRODUCTION OF 1082 MICROBIAL EXTRACTS

Fermentation conditions:

Each strain fermented in a “nutrient array” of ~**10** different culture media;
10 ml volume, EPA vials;
shaken 220 rpm, for 7,14 or 21 days, at 22 or 28 ºC, 70%HR.



Extraction procedures per 10 ml fermentations:

1x culture volume of acetone added;
EPA vials shaken for 2 hours;
Acetone evaporated in a N_2 flow and sample concentrated 2x the initial culture volume, in 20% DMSO.

Microplate preparation

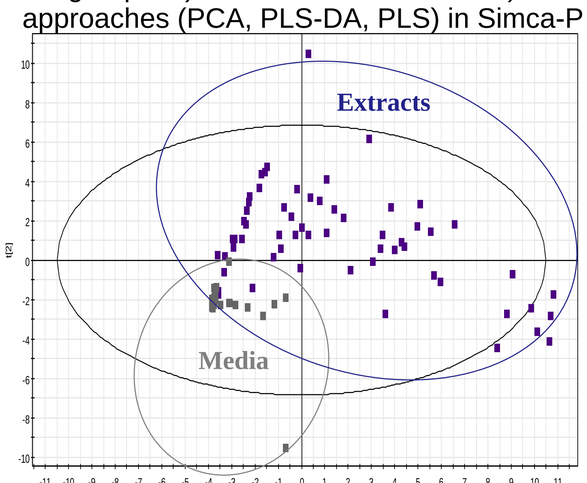
Extracts transferred to microplates with an automated sampler;
Extracts in microplates filtered through 1.2 µm (Fungal extracts) or 0.2 µm (actinomycetes) to eliminate biomass debris.

BIOEVALUATION: Microplate extracts were tested in HTS against the targeted assays (tyrosinase, elastase, collagenase, caseinase, antioxidant and cytotoxic activity):

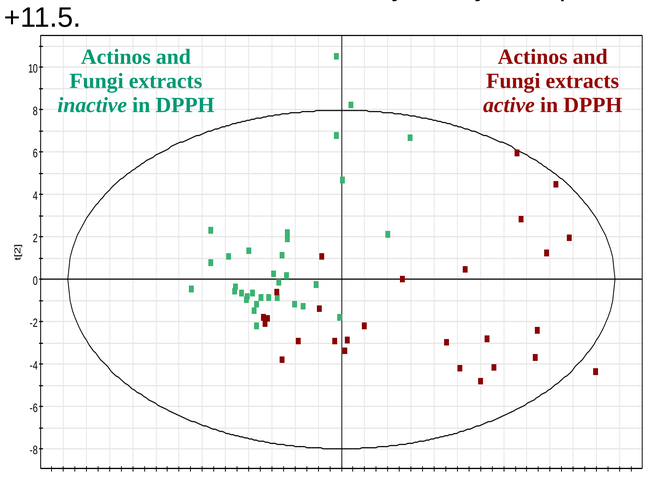
Initially 1082 extracts
104 active non-toxic extracts:
29 from fungal extracts
75 from actinomycetes extracts

RESULTS - METABOLOMICS

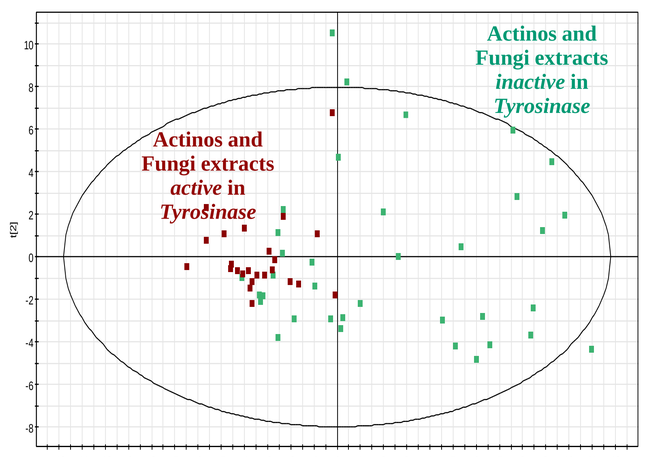
Analytical techniques: *Ultra Performance Liquid Chromatography – High Resolution Mass Spectrometry (UPLC-HRMS)* has been performed in (+) and (-) ionization modes, at 30000 resolution on an LTQ Orbitrap Discovery mass spectrometer. **Statistical Analysis**: Samples were divided initially into two groups: a) the Extracts and b) the Media samples. The Extracts were divided further into two subgroups: a) the active extracts and b) the inactive extracts. Data were analyzed by the open sources software MZmine 2.14.2 and XCMS package in R language. All data analyzed using Multivariate approaches (PCA, PLS-DA, PLS) in Simca-P +11.5.



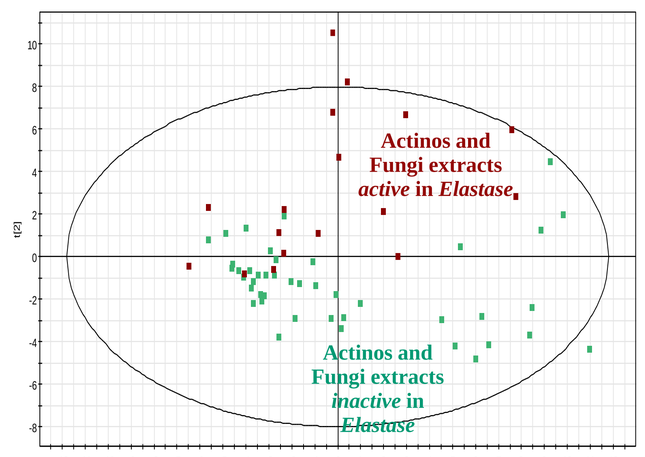
PLS-DA scores plot (UV scaled) of Extracts and Media samples, obtained by UHPLC-(+)ESI-HRMS. The Extract samples (purple boxes) and the Media samples (grey boxes) have the tendency to form two separate classes/groups.



PLS-DA model from Actinos and Fungi extracts, obtained by UHPLC-(+)ESI-HRMS, showing all the analysed samples (scores) with **DPPH activity** above threshold (red boxes) and below threshold (light green boxes)

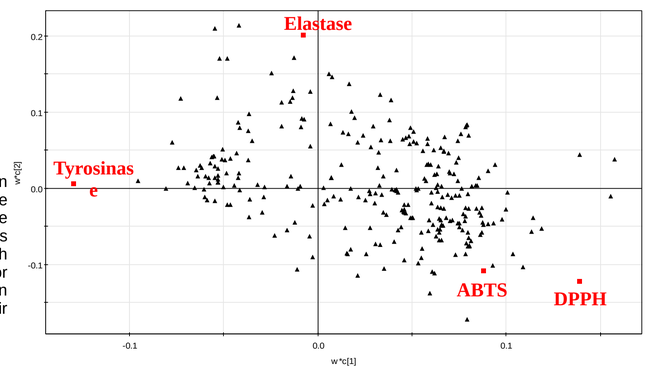


PLS-DA model from Actinos and Fungi extracts, obtained by UHPLC-(+)ESI-HRMS, showing all the analysed samples (scores) with **Tyrosinase activity** above threshold (red boxes) and below threshold (light green boxes)



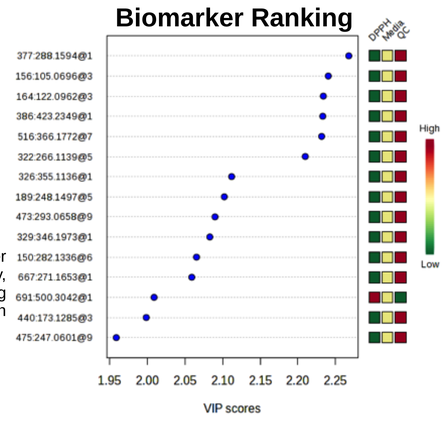
PLS-DA model from Actinos and Fungi extracts, obtained by UHPLC-(+)ESI-HRMS, showing all the analysed samples (scores) with **Elastase activity** above threshold (red boxes) and below threshold (light green boxes)

Loading Scatter Plot w*c1 *vs* w*c2 of PLS, displaying the major relation between the X’s (variables) and the Y’s (biological responses). Figure shows how the X-variables correlate with the Y-variables, and the correlation structure of the X's and Y's. X-variables with large w*'s (positive or negative) are highly correlated with Y. These variables with large w*'s are situated far away from the origin (on the positive or negative side) on the plot. Hence it is clear how the responses vary in relation to each other, which ones provide similar information and their relationship to the terms in the model.



Biomarker Discovery

The variable importance in the projection (VIP) parameter indirectly reflects the correlation of the metabolites with the activity, and is a widely used method for biomarker selection (according “greater than one rule”, the variables with VIP values larger than one will be selected for structural identification)



CONCLUSIONS

•From 1082 microbial extracts generated under “nutritional arrays” and tested for their cosmeceutical potencial, 104 were bioactive and non toxic. Those extracts were forwarded for an LC-HRMS based metabolomics analysis that showed clear differentiation of metabolites in extracts that are responsible for the detected bioactivity. Selected representatives of each cluster have been selected and are being cultivated in large scale bioreactors for isolation and identification of bioactive molecules.

•The microbial library of +116.000 strains of FUNDACIÓN MEDINA, is being explored for the first time in the frame of MICROSMETICS EU project for the discovery of novel cosmeceutical agents. The application of “nutritional arrays", the high throughput microbial extract preparation, the HTS screening in combination with LC-HRMS based metabolomics seems to be a very effective method for the discovery of novel metabolites from microbial extracts with applications in cosmeceutical industry.

SEVENTH FRAMEWORK PROGRAMME

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