RATIONAL DRUG DESIGN TOOLS FOR THE DISCOVERY OF NOVEL MICROBIAL NATURAL PRODUCTS WITH APPLICATIONS IN COSMETICS

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INTRODUCTION

Cosmetics are important consumer products with an essential role in human's life. Besides traditional natural cosmetics derived from plant extracts, there are many new products based on synthetics ingredients. Since cosmetics products have a lifespan of less than 5 years, manufacturers reformulate ¼ of their products every year to improve products constantly and stay ahead in a highly competitive market for better efficacy. Conventional animal testing for chemical ingredients used in cosmetics is prohibited. Moreover, in United States, the US Environmental Protection Agency (US EPA) discourages the use of animal testing even for toxicity estimation. In Europe, the European Parliament & Council of the European Union have prompted the development of alternative methods that assess skin sensitizing potential of chemicals. Since *in-vivo* animal testing is prohibited, other strategies are urgently needed. In-vitro experiments combined with (Q)SARs or/and in-silico studies could be the magic bullet to pass this dead-end.

Cosmetics products can act based on their physicochemical properties (UV filters, colorants, etc.) or by binding to a specific receptor. Since biology has augmented the number of known human skin's proteins, those proteins can be handled as drugable targets. Using common Rational Drug Design Tools such as Similarity Search, *In-Silico* Screening and Prediction Models we can rationalize the bioprospection of natural sources producing appropriate metabolites for cosmetic use (**Figure 1**). In the frame of the EU project "MICROSMETICS", we exploit the microbial global biodiversity to discover novel cosmeceutical agents by developing a specific Prediction Model and *in-silico* Screening against four specific cosmeceutical validated targets, namely Elastase, Collagenase, Tyrosinase and Hyaluronidase.

METHODS

Functional Prediction Model

Starting from the CosIng Repository, accurate functional prediction model was created for all known cosmetic functions. From the **21579** records we successfully retrieved **6206** organic molecules. Those molecules were annotated to specific functions and finally we created a prediction model for all 65 available functions. This model is included in the upcoming version of **CTlink** software (www.chemotargets.com).

Database of Microorganism Metabolites

ANTIBASE 2012 containing 40000 records, was selected as input of all known metabolites from microorganism and higher fungi. All molecules were fully prepared for Virtual Screening using the LigPrep protocol of Schrodinger Suite 2014 (Schrodinger Inc) and the default workflow of OPENEYE suite for the Similarity search.

Similarity Search

Exploring the BindinDB Database, we selected all molecules with Ki or IC_{50} less than 1μM for Tyrosinase, Elastase and Collagenase. Hyaluronidase lacks of known binders so it was excluded from the similarity search. From all ligands found we created a diversity set of 20 molecules which was used as query dataset. All molecules from the prepared Antibase database was passed through similarity search for each query molecule.

In-Silico Virtual Screening

Protein Preparation

For Tyrosinase, Hyaluronidase 1 and Collagenase in-silico screening the following PDB IDs structures were chosen: 2Y9X, 2PE4 and 2Y6I respectively. For Elastase we couldn't find appropriate template in PDB, so we performed homology modeling taking as template the PDB ID 1ELT using Prime Software (Schrodinger Inc). The sequence alignment was performed using ClustalW as implemented in Prime software. Finally all 4 receptors passed through Protein Preparation Wizard as implemented in Schrodinger Suite 2014 prior to virtual screening (**Figure 2**).

Virtual Screening

The resulted from LigPred protocol ANTIBASE molecules, were filtered through virtual screening procedure using Glide software (Schrodinger Inc) and High Throughput Virtual Screening (HTVS) protocol. For each target we collected all molecules with theoretical free energy of binding (GlideScore) less than 2Kcal/mol from the top hit molecule.

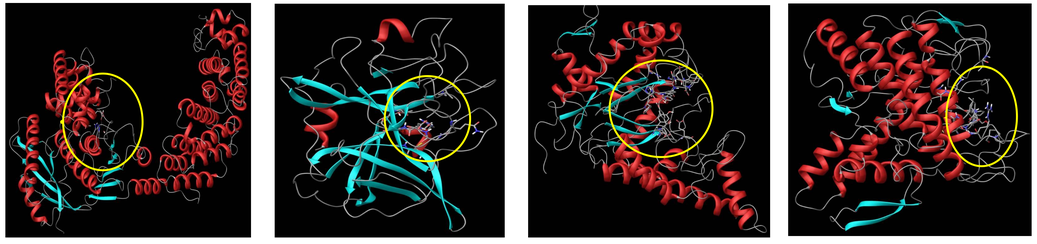

Figure 2. 3D structure of Collagenase, Elastase, Hyaluronidase and Tyrosinase used in Virtual Screening. Binding cavity is marked with yellow circle.

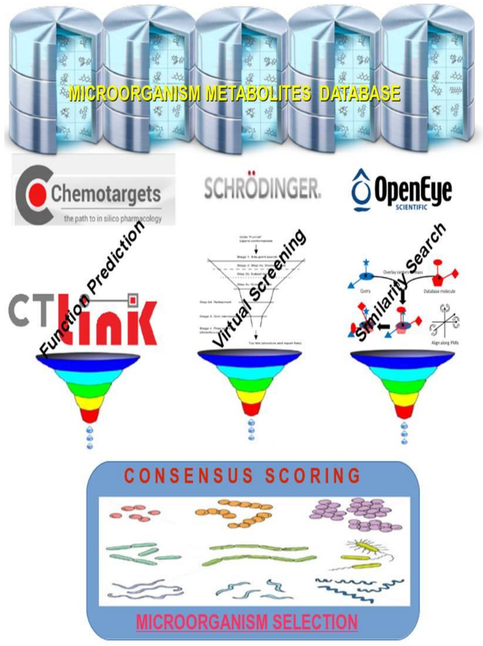

Figure 1. Workflow of Rational Discovery of Novel Microbial Natural Products for Cosmetic use.

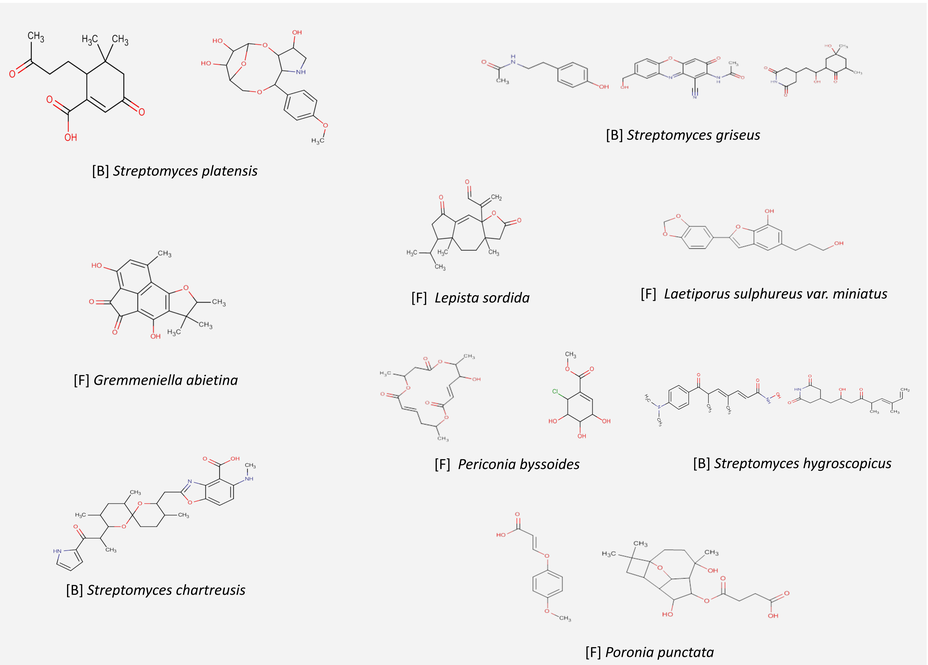

Figure 3. Most promising hit molecules and their Fungi or Bacterial source

RESULTS

Consensus Scoring Filtering

All data collected from Functional Prediction, Similarity search and Virtual Screening was gathered in order to create a consensus scoring filter for final microorganism selection.

From Functional Prediction Model 1480 metabolites were correlated with at least one cosmetic function using CTlink software and on the same time they passed the safety prediction model of CTlink software.

From virtual screening, 93 metabolites ranked on the first 10% on all 4 receptors, 125 metabolites on 3 receptors and 197 metabolites on 2 receptors.

From similarity search 93 metabolites ranked on the top 10% for 3 targets (hyaluronidase lacks of known binders), 242 metabolites for 2 targets and 470 metabolites for 1 target.

Combining all the above results we selected 100 strains of microorganisms for which metabolites found on the top hits on all three protocols (functional prediction model, Similarity search and Virtual screening). In **Figure 3** we present the most promising hit molecules for all or some of the targets of interest together with the source Bacteria of Fungi. .

PROSPECTIVES

The selected strains have been cultivated under "nutritional arrays", extracted. From the 100 selected strains more than 1000 extracts have been generated and are currently tested in vitro in order to be prioritized for the isolation and identification of possible novel bioactive molecules with application in cosmetic industry. The results so far are very promising, validating our *in-silico* rational prediction.

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