Development of a miniaturised screening method for fungal mutants with enhanced production of specific natural compounds

Annemarie Kramer[1)], Linda Paun[2)], Antje Labes[1)], Frank Kempken[2)] and Johannes F. Imhoff[1)]

[1)]*Kiel Centre for Marine Natural Products at GEOMAR, Am Kiel-Kanal 44, D-24106 Kiel, Germany*

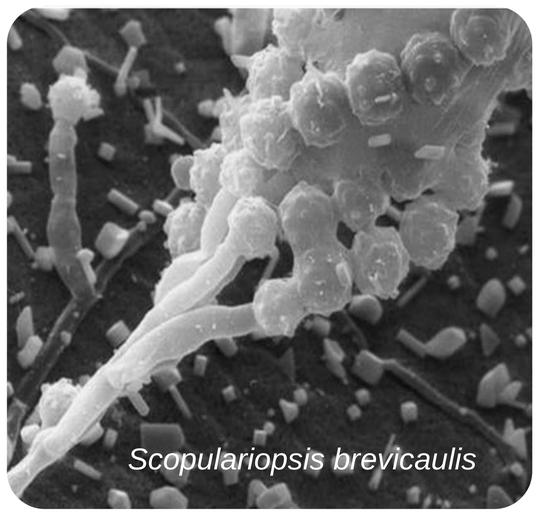
[2)]*Department of Genetics & Molecular Biology in Botany, Institute of Botany, Christian-Albrechts-University at Kiel, Ohlshausenstraße 40, D-24098 Kiel ,Germany*

KiWiZ Kieler Wirkstoff-Zentrum am GEOMAR

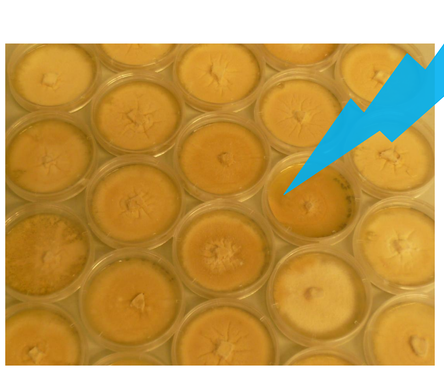
MARINE FUNGI

Introduction

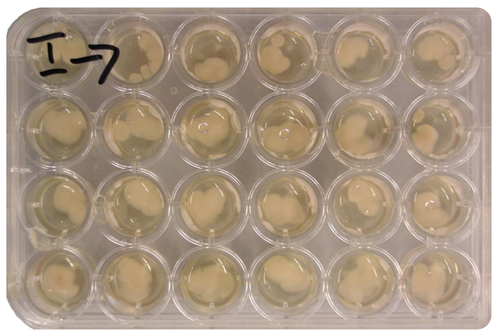
Within the EU-project MARINE FUNGI (EU FP7, 265926), the marine fungus *Scopulariopsis brevicaulis,* isolated from the marine sponge *Tethya aurantium*, was selected for a molecular optimisation process of its secondary metabolite production. Using random mutagenesis by UV radiation, the production of the two cyclodepsipeptides scopulariade A and B [1] should be enhanced. A challenge during this molecular optimisation process was the handling of the huge number of mutants, whose secondary metabolites are not easily detected, as e.g. by visual control or antibiotic activity determination. Hence, the identification of the secondary metabolites of each mutant strain is still a time and material consuming step. Therefore, a miniaturised screening method was developed. The established method covers a decreased cultivation volume, a fast extraction method and an optimised LC-MS analysis format. With this method, a remarkable time reduction could be achieved and in addition, a reduction of process deviation, important for the comparability of the screening results.



Scopulariopsis brevicaulis

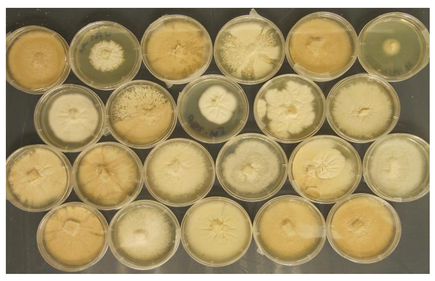


Random mutagenesis achieved by UV-radiation with a survival rate of 1 %



Cultivation on 24-well plate, containing 1 ml of culture media

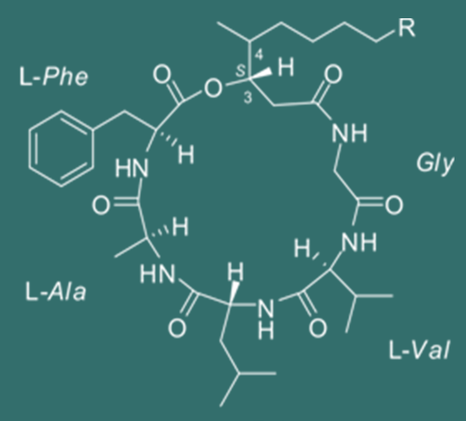
Establishing of a mutant library



Fast extraction

Because of the decreased cultivation volume, only 1 ml of organic solvent is required and a high extraction through-put is possible.

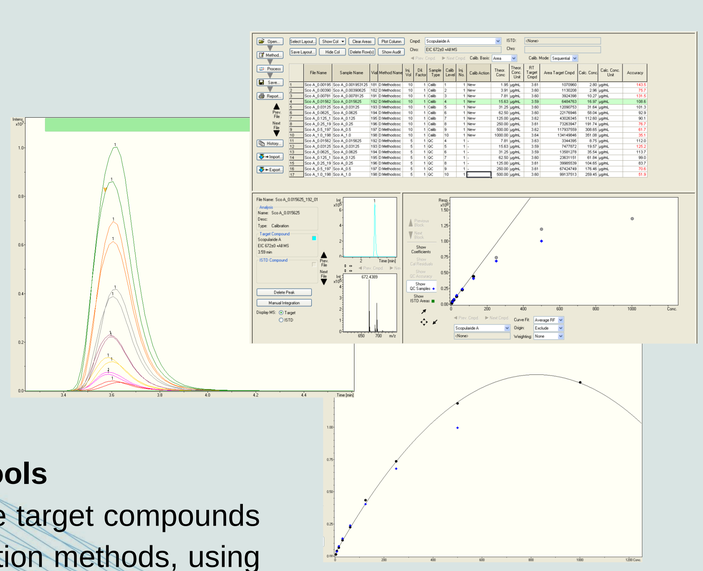
Scopulariade A/B [1], the target molecules within this screening, are not detectable by visual control or antibiotic activity.



Scopulariade A: R = C_2H_5
Scopulariade B: R = H

LC-MS system for determination of production rate compared to dry biomass

Use of a mircOTOF II Bruker Daltonics combined with a VWR Hitachi Elite LaChrom system for determination of production rate per dry biomass

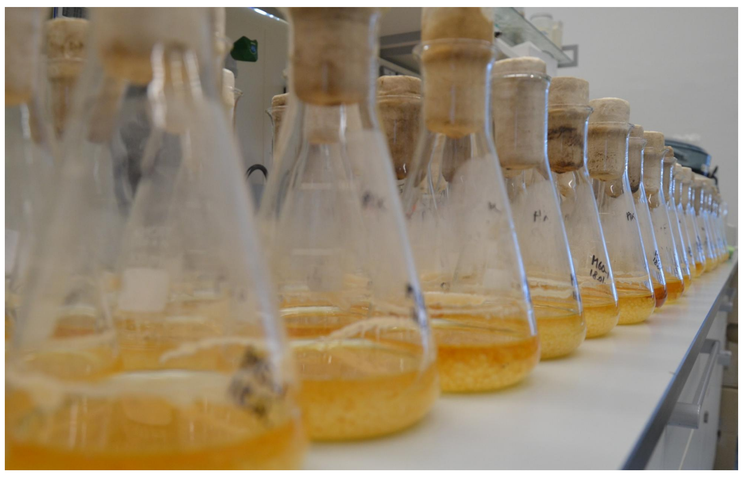


Use of quantification software tools

Determination of the amount of the target compounds scopulariade A and B via quantification methods, using the QuantAnalysis software of Bruker.

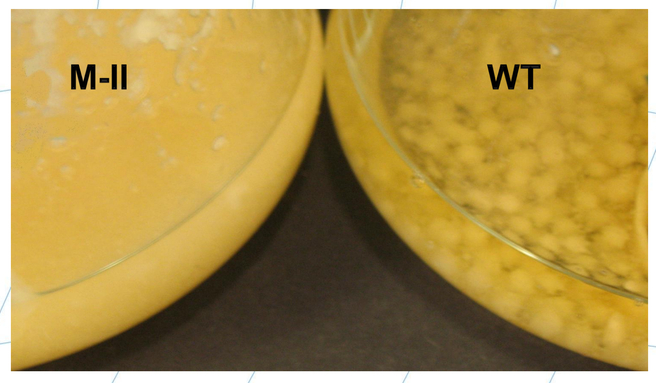
Selection for 100 ml approach

Due to the effective screening in 1 mL scale, an enormous reduction of number of mutant strain for throughout characterisation was possible: the best producers correlate to approximately 10 % from all strains sampled at 1 ml. The 100 mL approach could be done easily in duplicates, because of the lower sample number at this step.

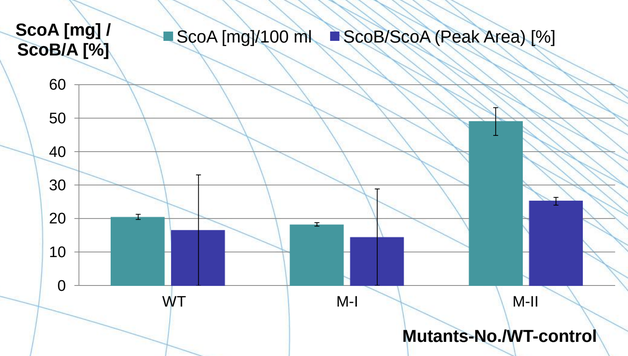


Enhanced production of selected mutant strain M-II

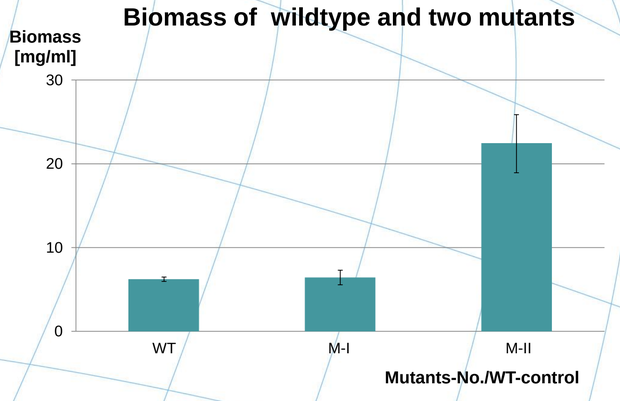
For a detailed characterisation of the selected mutants, the amount of scopulariade A and B and the biomass of the strains were compared. For mutant strain M-II a modified morphological growth and a higher biomass production resulting in a higher yield of scopulariade A and B could be detected. This modification may be of advantage in biotechnological handling in stirred tank reactors.



Amount of Scopulariade A [mg] and ratio of B to A [%]



Biomass of wildtype and two mutants



MARINE FUNGI

SEVENTH FRAMEWORK PROGRAMME

Fungi are well known as good producers of natural compounds. However, the potential of marine fungi to produce bioactive compounds is under investigated. To improve this knowledge, the EU-project **MARINE FUNGI (EU FP7, 265926)** has set its focus on the isolation and characterisation of new anticancer compounds from marine fungi. To improve the production of the compounds or even to change the compound spectra, diverse methods are used within the project.

For further information visit: **www.marinefungi.eu**
Or see poster number **IBP013** or join talk **GMV006**.

References

[1] Yu, Z.; Lang, G.; Kajahn, I.; Schmaljohann, R.; Imhoff, J. *J. Nat. Prod.* **2008**, *71,* 1052–1054