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

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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 cyclosporins epidesides scopularide 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.

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

Enhanced production of selected mutant strain M-II
For a detailed characterisation of the selected mutants, the amount of scopularide 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 scopularide A and B could be detected. This modification may be of advantage in biotechnological handling in stirred tank reactors.

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.

References