Higher yields of cyclodepsipeptides from *Scopulariopsis brevicaulis* by random mutagenesis

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

1. Department of Genetics & Molecular Biology in Botany, Institute of Botany, Christians-Albrechts-University at Kiel, Olshausenstr. 40, D-24098 Kiel, Germany [fkempken@bot.uni-kiel.de; lpaun@bot.uni-kiel.de]

2. Kieler Wirkstoff-Zentrum KiWiZ at Geomar, Am Kiel-Kanal 44, D-24106 Kiel, Germany

Introduction

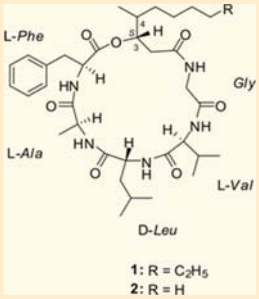
The ascomycete *Scopulariopsis brevicaulis* produces two cyclodepsipeptides, scopularides A and B [1], which show activity against several tumor cell lines. Within the EU project MARINE FUNGI (EU FP7, 265926) one of our aims is to enhance the production of these secondary metabolites. We established two ways of random mutagenesis. We created a UV-mutant library and screened the mutants. We developed a miniaturised screening method and were able to identify several mutants with a higher scopularide production in comparison to the wild type. One of these mutants produces three times more biomass and more than double the amount of scopularide A. Next Generation Sequencing is being employed to identify the molecular genetic basis of the observed mutations. In parallel we employ transposable elements to introduce mutations [2]. The impact of transposons on gene expression as well as their ability to cause major mutations makes them an interesting tool for random mutagenesis [3, 4, 5]. We employ the *Vader* transposon in its homologous host and found that *Vader* mostly integrates within or very close to genes. Thus it appears to be a useful tool for transposon-mediated mutagenesis in *A. niger* [6].

EU project MARINE FUNGI

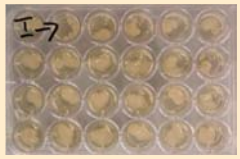
Within the EU project (EU FP7, 265926) fungal strains of marine origin are being utilized as producers of anti-cancer drugs. Out of a huge library of fungi three of them are chosen to be further analyzed regarding their production of secondary metabolites. *Scopulariopsis brevicaulis* was chosen as the first candidate.

Scopulariopsis brevicaulis

This ascomycete was isolated from the sponge *Tethya aurantium*. It produces the secondary metabolites Scopularide A and B [1]. These Cyclodepsipeptides show activity against several tumor cell lines like Panc89, HT29 and Colo357.



Mutant-Screening



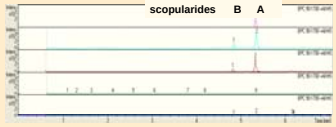
cultivation in 1 mL liquid medium

↓

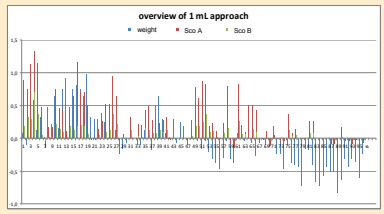
isolate scopularides A and B with EtOAc in SpeedMill

↓

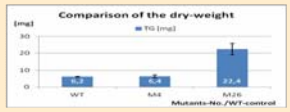
analyze extracts with mass spectrometry



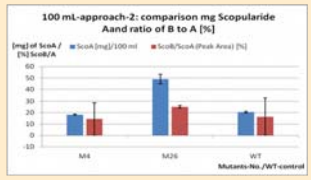
Chromatogram of 5 different extracts



Comparison of data achieved with 1 mL culture



Comparison of the mutant M26 to the wild type shows the potential as a high producer of ScoA & B



Conclusions

- Miniaturised screening method led to the identification of the mutant M26
- M26 is more suitable for high fermentation and is faster growing than the wild type

Future prospects

→ Next Generation Sequencing is being performed to identify mutations

→ establish transposon based mutant library

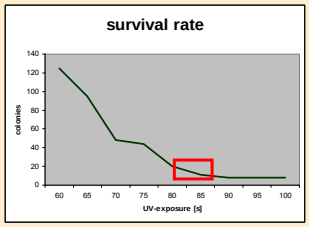
→ establish mutant libraries with candidate 2 and 3

MARINE FUNGI

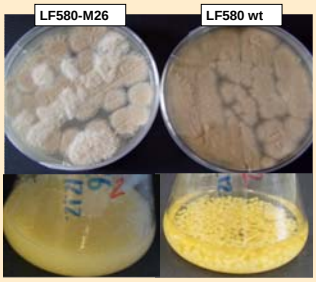
www.marinefungi.eu

UV-Mutagenesis

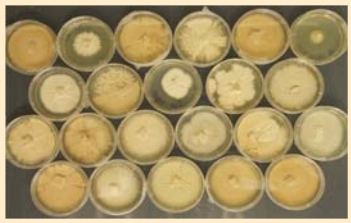
UV-radiation was performed on 2000 isolated conidia for 85 sec (1.17 kj/m²). The survival rate was set to 1 %.



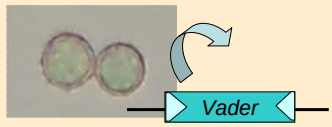
A mutant library was established and screened for higher production of ScoA and B.



Mutant M26 was used for a second round of mutagenesis.

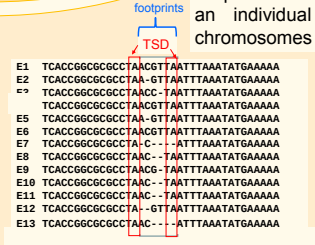


Transposon-Mutagenesis



The *Vader* element was successfully tested as a mutagenesis tool in *Aspergilus niger*.

Sequence analysis of 13 excision events: each exhibition shows an individual footprint. Reintegration of *Vader* occurs in chromosomes I, II, III, IV, V and VIII (orange and red spots). The original integration site is on chromosome VIII (blue spot).



Vader is a non-autonomous transposon. The *Tan1-Vader*-cassette was developed for the usage in heterologues hosts such as *S. brevicaulis*.

References

[1] Yu, Z.; Lang, G.; Kajahn, I.; Schmaljohann, R.; Imhoff, J. *J. Nat. Prod.* **2008**, *71*, 1052–1054
[2] Braumann I, van den Berg M, & Kempken F (2007) *Fungal Genet Biol* 44(12):1399-1414.
[3] Daboussi MJ & Capy P (2003) *Annu Rev Microbiol* 57:275-299.
[4] Kempken F (2003) *Applied Mycology and Biotechnology, Vol. 3 Fungal Genomics*, eds Arora DK & Khachatourians GG (Elsevier Science Annual Review Series), pp 83-99.
[5] Kempken F & Kück U (1998) *BioEssays* 20:652-659.
[6] Hihlal E, Braumann I, van den Berg M, Kempken F (2011) *Appl Environment Microbiol*, 77: 2332–2336

Acknowledgements

Part of the work was funded by the DSM Anti-Infectives.
L.P. and E.H. received a fellowship of the Max-Buchner-Stiftung.
Visit us in the web: **http://www.uni-kiel.de/Botanik/Kempken/fbkem.shtml**