TECHNOLOGY FOR BUSINESS

Scopularide A production from the Marine Fungus *Scopulariopsis brevicaulis*

Anu Tamminen, Yanming Wang and Marilyn Wiebe

VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044, VTT, Finland

Introduction

Fungi isolated from marine environments are good sources of unique bioactive compounds. *Scopulariopsis brevicaulis*, isolated from a marine sponge by researchers in Johannes Imhoff's group at the Kieler Wirkstoffzentrum in Germany, produces cyclodepsipeptides which may have anti-tumour activity. Scopularide A is one such cyclodepsipeptide (Yu *et al.,* 2008). It accumulates within the hyphae of *S. brevicaulis* LF580.

Little is known about the induction or regulation of scopularide A accumulation in the hyphae of *S. brevicaulis* (Fig. 1), or the conditions necessary for production. Unlike many secondary products, scopularide A is produced during growth.

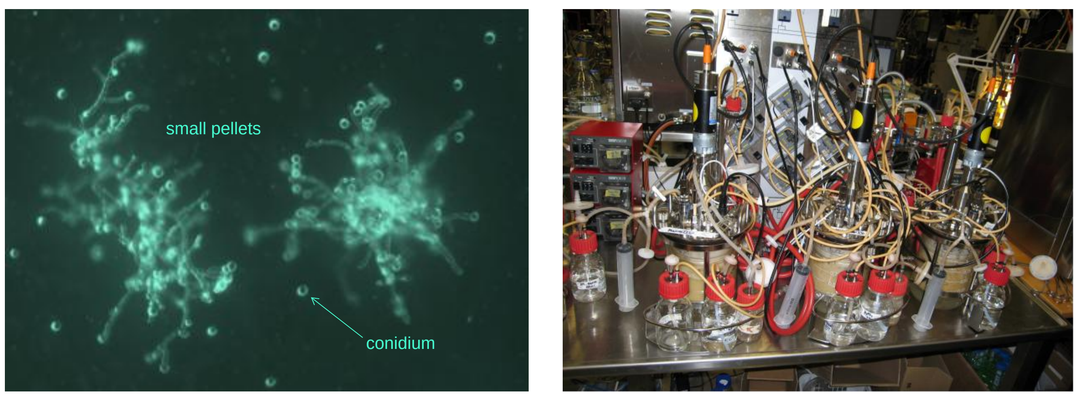


Fig. 1. *Scopularide brevicaulis* LF580 (left, producing conidia and small pellets) in 1 L BiostatQ bioreactors (right) produces scopularides on modified Wickerham medium. Cultures were inoculated with 10% (v/v) filamentous pre-cultures which were generated in flasks containing medium with 4 g/L agar, inoculated with 2x10^5 spores/ml.

Results

- Scopularide A was produced in 0.5 to 10 L bioreactors. Production was dependent on provision of adequate carbohydrate, nitrogen and air (Table 1).
- Both complex (Wickerham) and defined (Vogel, 1956) media with 3% sea salt supported good scopularide A production (Table 1). Corn steep solids could replace yeast extract and peptone.
- Low pH and poor stirring/aeration reduced scopularide A production. Salt concentration, however, did not appear critical for scopularide A production.

Table 1. Biomass and scopularide A production by *S. brevicaulis* LF580 in bioreactor cultivations at pH 7, with 3% marine salt, 28 C.

Condition	Glucose (g L^{-1})	C/N (g g^{-1})	Biomass (g L^{-1})	Yield$_{x/S}$ (g g^{-1})	Scopularide A (mg L^{-1})
Static flask (Yu et al., 2008)	10	9			7
O_2-limited batch	11	9	11	0.79	10
Batch	11	9	11 ± 1	0.78	29
N-limited batch	21	14	12 ± 1	0.51	8
Batch	20	11	15 ± 2	0.63	58
Batch or fed-batch	44	10	25 ± 1	0.56	128
Batch or fed-batch	61	9	33 ± 1	0.54	162
Batch – CSS	17	7	12	0.68	44
Batch - defined	17	9	12	0.68	41
Batch - defined	40	9	24 ± 6	0.59	44
Batch – defined, xylose	40	9	25 ± 1	0.64	54

References

1. Schulz D, Ohlendorf B, Zinecker H, Schmaljohann R, Imhoff JF. 2011. Eutypoids B-E produced by a *Penicillium* sp. strain from the North Sea. *J.Nat. Prod. 74,* 99-101.
2. Vogel HJ. 1956. Convenient growth medium for *Neurospora* (Medium N). *Microb Genet Bull* 243, 112-119.
3. Yu Z, Lang G, Kajahn I, Schmaljohann R, Imhoff JF. 2008. Scopularides A and B, cyclodepsipeptides from a marine sponge-derived fungus, *Scopulariopsis brevicaulis*. *J. Nat. Prod. 71*, 1052-1054.

Results

- Biomass production was increased by increasing the glucose concentration using either batch or fed-batch processes, resulting in increased scopularide A production (Fig. 2).
- Surprisingly, not only volumetric, but also specific scopularide A production (mg scopularide [g biomass]$^{-1}$) was increased in high cell density cultures (Fig. 3).
- Initial productivity was higher in batch than in fed-batch cultures, but the total amounts produced were similar (Fig. 4).
- These studies provide a basis for developing an improved production process.

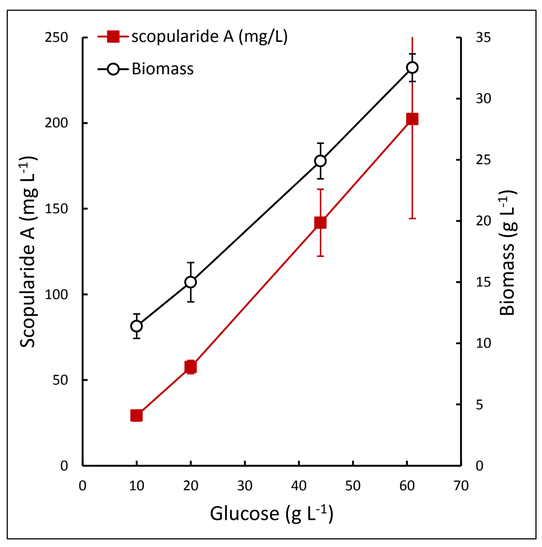


Fig. 2. Production of biomass and scopularide A in batch or fed-batch fermentations. *S. brevicaulis* LF580 was grown in modified Wickerham medium with 3% Sea Salt at pH 7, 28°C, >30% DOT.

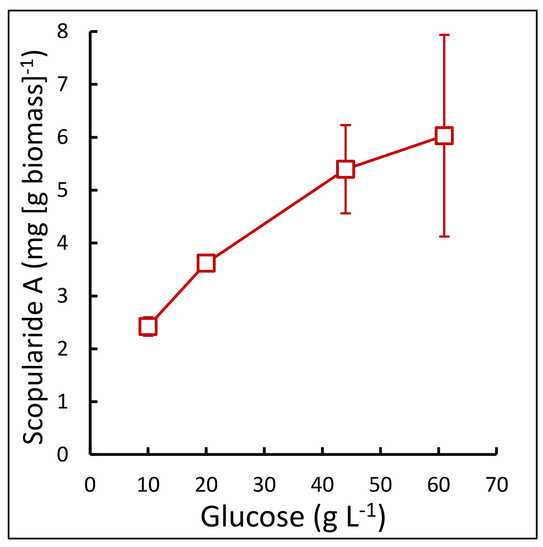
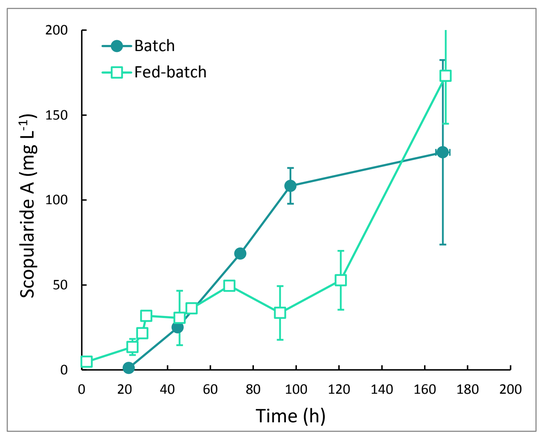
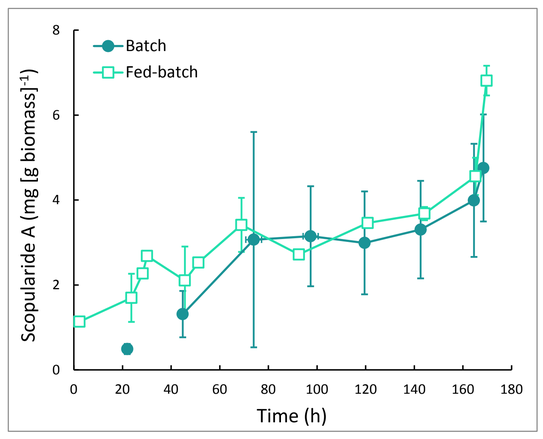


Fig. 3. Specific scopularide A production by *S. brevicaulis* LF580 (in modified Wickerham medium with 3% sea salt at pH 7, 28°C, >30% DOT, batch or fed-batch cultures).





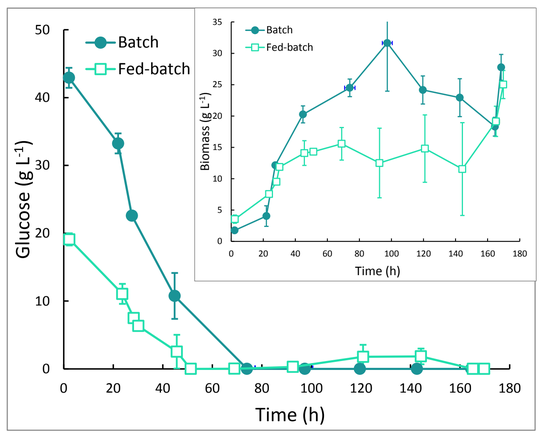


Fig. 4. Production of scopularide A (upper left) and biomass (lower inset) in fed-batch (open symbols) and batch fermentations (solid symbols) shows that although the final amount of scopularide A produced was similar, higher concentrations could be obtained earlier in the batch than in the fed-batch cultures. Specific scopularide A production (upper right) was not affected by the culture mode.

Conclusion

By using STRs to obtain well aerated high cell densities we successfully increased production of scopularide A by S. brevicaulis LF580 by approx. 29-fold compared to production in stationary flasks (Yu et al. 2008). We also demonstrated that scopularide A could be produced in defined medium.

- It is now possible to generate 500 mg scopularide A using less than 3 l culture.

SEVENTH FRAMEWORK PROGRAMME

ACKNOWLEDGMENTS

This study was financially supported by the European Commission through the Seventh Framework Programme (FP7-KBBE-2010-4) under grant agreement N° FP7-265926 MARINE FUNGI and is coordinated by Johannes F. Imhoff (KiWiZ, Kieler Wirkstoff-Zentrum am GEOMAR, Am Kiel-Kanal 44, 24106 Kiel, alabes@geomar.de, www.kiwiz.org).

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