The Production of Eicosapentaenoic Acid by *Mortierella Spp* on Barley Spent Grain

A JACOBS[1], LM DU PLESSIS[1], N NXUMALO[1] AND A COMBRINK[1]
[1]CSIR Biosciences, PO Box 395, Pretoria 0001
[1]Email: ajacobs@csir.co.za

SUMMARY
There is a growing interest in sources alternative to fish oil of long chain polyunsaturated fatty acids (PUFA) particularly the ω-3 PUFA's such as eicosapentaenoic acid (EPA). The current study aims to produce ω-3 PUFA's, by solid state fungal fermentation, on barley spent grain, a by product of the brewing industry and a food grade waste. The substrate has a high protein and fat content (21-25% and 9-10% respectively on a dry basis).

The enzymes used by the fungi to produce ω-3 PUFA's convert α-linolenic acid (ALA) into EPA. In this study the substrate was supplemented with linseed oil (containing approximately 60% ALA) to determine the effect of ALA on growth and PUFA-production.

METHODOLOGY
Fungal isolates of *Mortierella kuhlmanii, M. alpina, M. antarctica, M. sarnyensis* and *M. parvispora* were obtained from Dr A Botha (University of Stellenbosch). Stock cultures were held on Malt Extract Agar (MEA) slopes at 5°C.

Barley spent grain (BSG) substrate series was prepared with and without lipid supplementation (13% linseed oil), sterilised and inoculated with prepared homogenised inoculum. The series were incubated at 22°C for 3 days, followed by incubation at 12°C for 7 days. The fermented BSG was harvested, weighed and dried at 50°C for 4 days and analysed for the presence of PUFA. The amount of fungal growth in fermented BSG was determined by performing plate counts on MEA. Lipid extraction and determination of the fat content was done by the method of Folch (1957).

RESULTS AND DISCUSSION

Isolate No	Fungal Isolate	Fungal Growth (cfu/g)	
		BSG with no supplement	BSG with 13 % LSO supplement
Mo 018	*Mortierella kuhlmanii*	2,0 x10^3	3,0 x 10^2
Mo 038	*M alpina*	4,5x10^4	8,5 x 10^4
Mo 059	*M antarctica* (4g)	2,0x10^4	1,2 x 10^5
Mo 064	*M sarnyensis* (4n)	3,0 x10^3	ND
Mo 074	*Mortierella spp* (5e)	2,0 x 10^1	1,1 x 10^2
Mo 077	*M alpina* (5h)	4,0 x 10^1	9,5 x 10^2
Mo 081	*M parvispora* (9c)	4,5 x 10^1	<10
Mo 085	*M antarctica* (9g)	3,5 x 10^1	5,0 x 10^2
Control	BSG, not inoculated	<10	<10

Cfu/g = colony forming units per gram
ND = not done

Table 1: Growth of 8 fungal strains on barley spent grain (BSG) supplemented with 13% linseed oil (w/w)

Three of the fungal strains grew better on spent grain supplemented with 13% linseed oil, while 4 other fungal strains grew better on BSG with no supplement. The addition of linseed oil to the substrate did therefore not appear to have an influence on the growth of the fungi or the colonisation of the BSG (**Table 1**).

Figure 1: Barley spent grain inoculated with EPA-producing fungal isolate (left) and un-inoculated control (right)

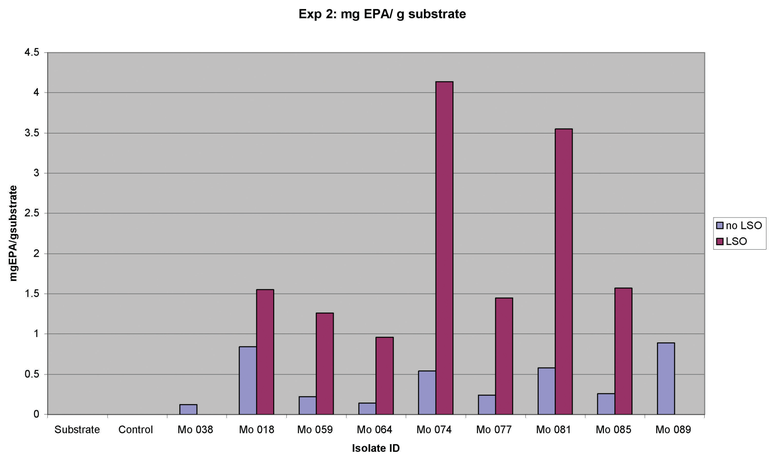

Figure 2: Production of EPA on dried BSG by 9 fungal strains (mg EPA/g substrate on a dry basis)

The production of PUFA was enhanced by the addition of linseed oil (**Figure 2**).
Mo 018, Mo 059, Mo 064, Mo 077, Mo 081 and Mo 085 produced considerably more EPA when the ω-3 precursor was added to the substrate. The production of EPA by Mo 074 (*Mortierella spp*) was increased from 0,5 mg to 4,1 mg EPA per g of substrate (on a dry base) with the addition of 13% linseed oil.

CONCLUSION
According to the results of this study long chain PUFAs and EPA can be produced on dried barley spent grain by solid state fungal cultivation. The production of EPA is enhanced by the addition of linseed oil to the substrate.

BIBLIOGRAPHY
1. Bajpai, P, Bajpai, PK and Ward, OP, 1991a. Eicosapentaenoic acid (EPA) production by *Mortierella alpina* ATCC 32222. *Applied biochemistry and biotechnology*. **31** pp. 267–272
2. Botha, A, Strauss, T, Kock, JLF, Pohl, CH and Coetzee, DJ, 1996. Carbon sources utilization and γ-linolenic acid production by Mucoralean fungi. *System applied microbiology*. **20** pp. 165-170
3. Certik, M and Shimizu, S, 1998. Biosynthesis and regulation of microbial polyunsaturated fatty acid production. *Journal of bioscience and bioengineering*. **87**(1) pp. 1–14
4. Cheng, MH, Walker, TH, Hulbert, JG and Raman, RD, 1998. Fungal production of eicospentaenoic and arachidonic acids from industrial waste streams and crude soybean oil. *Bioresource technology*. **67** pp. 101–110
5. Naylor, RL, Goldburg, RJ, Primavera, J, Kautsky, N, Beveridge, MCM, Clay, J, Folke, C, Lubchenco, J, Mooney, H and Troell, M, 2001. Effects of aquaculture on world fish supplies. *Issues in ecology*. **8** pp. 1–12
6. Stredansky, M, Conti, E and Salaris, A, 1999. Production of polyunsaturated fatty acids by *Pythium ultimum* in solid state cultivation. *Enzyme and microbial technology* **26**(2000) pp. 304–307
7. Zhu, H, 2002. Utilization of rice bran by *Pythium irregulare* for lipid production. MSc biological and agricultural engineering thesis, Zhejiang University, Zhejiang.