DETERMINATION OF HYDROCARBONS IN FUNGI GROWING MEDIA BY TWISTER TDU-GC/Q-TOF

Camilla Liscio, Anatune Ltd., Girton, Cambridgeshire, UK.

INTRODUCTION

Microbial conversion of cellulosic biomass to fuels or chemicals has the potential to mitigate our dependence on petrochemicals. Many fungi have been reported to produce a variety of hydrocarbons as metabolites which could be used as biofuels or chemicals. In this study, Twister® stir bars were deployed in fungal cultures to monitor the production of hydrocarbons and related metabolites. Twister® (Stir Bar Sorptive Extraction) is a very efficient enrichment technique which uses PDMS coated stir bars to perform sorptive extraction of the sample which is then thermally desorbed using the GERSTEL Thermal Desorption Unit (TDU) and analysed by GC/MS.

METHODS

Sample preparation:
Conditioned PDMS Twisters® with 0.5 mm film thickness x 10 mm length were immersed and stirred in fungi growing media over the last 24 hours of growth before quenching. Once retrieved, Twisters® were rinsed with MQ water, dried on lint-free tissue and transferred to TDU tubes for desorption.
Sample list: 1 background, 1 Control, 4 replicates of isolate 309.71 of *Ascocoryne sarcoides*, 4 replicates of isolate 246.80 of *Ascocoryne sarcoides*.
Instrumentation
<u>*Autosampler:*</u> GERSTEL MPS Robotic Dual Head, USM Tool equipped with gripper for TDU tube handling
<u>*Modules:*</u> VT40 TDU tubes Tray
<u>*GC-MS:*</u> GERSTEL TDU-CIS injector, Agilent GC 7890- Q-TOF 7200, Removable Ion Source (RIS), EI mode. Mass range: 50-650 *m/z*

RESULTS

Twister® samples were analysed in random order by TDU-GC-MS. Data were deconvoluted and library searched using Agilent Mass Hunter Unknown Analysis. Table 1 summarises the components identified as hydrocarbons appearing consistently across all analysed samples for the two fungi species. Compound 8 low match factor was due to the lack of match in the low molecular weight region not acquired on the MS (<50*m/z*). Figure 1 shows a Total Ion Chromatogram (TIC) of a Twister sample and the extracted ion chromatogram (EIC) for two of the identified compounds.

Compound ID	RT [min]	Formula	Match Factor
1	10.691	C_8H_8	91
2	35.700	$C_{16}H_{34}$	80
3	41.700	$C_{31}H_{64}$	95
4	31.687	$C_{15}H_{24}$	68
5	35.500	C_9H_{18}	69
6	24.000	$C_{12}H_{24}$	82
7	27.426	$C_{15}H_{24}$	85
8	24.443	$C_{15}H_{28}$	67

Table 1: Identified hydrocarbons across all Twister samples

1:Styrene **2:** Hexadecane **3:** Hentriacontane **4:** Aristolochene **5:** Cyclohexane, (1-methylethyl)- **6:**1-Undecene, 7-methyl- **7:** .alpha.-Muurolene **8:** Naphthalene,decahydro-1,4a-dimethyl-7-(1-methylethyl)-,[1S(1.alpha.,4a.alpha.,7.alpha.,8a.beta.)]

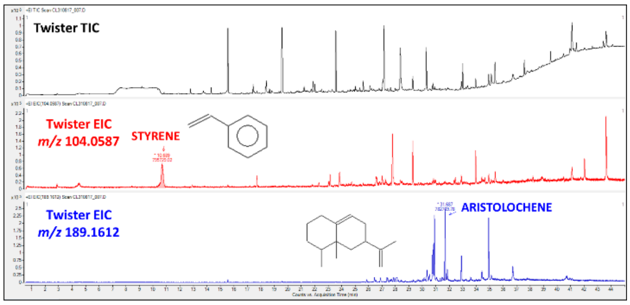

Figure 1: TIC and EIC for a Twister sample

4 compounds (1, 4, 7, and 8) showed significant presence across all replicates. Figure 2 summarises the average areas and standard deviation for the selected compounds in the control (grey) and in the fungi (AS309 orange, AS246 green). Error bars are 1 SD.

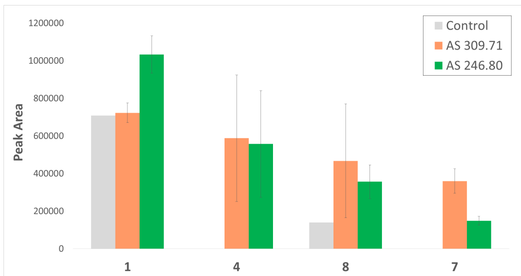

Figure 2: Average areas for the most significant hydrocarbons.

CONCLUSIONS

A range of hydrocarbons could be detected only in fungi growing media and not in the control sample using Twister® as enrichment technique.

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Dr Thomas Howard, Senior Lecturer, University of Newcastle
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Anatune Ltd
Unit 4, Wellbrook Court, Girton Road, Cambridge, CB3 0NA, UK
Tel: +44 (0) 1223279210 Fax: +44 (0) 1223279253
Email: info@anatune.co.uk Internet: www.anatune.co.uk