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Electronic Supplementary Information

Multi-well fungal co-culture for de novo metabolite-induction in time series studies based on untargeted metabolomics

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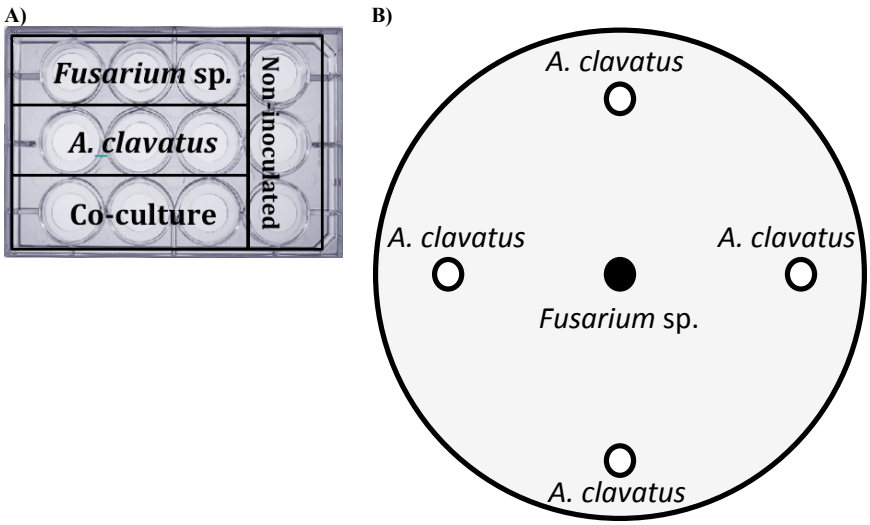

Fig. S1. Organization and inoculation of Petri dishes; A) miniaturized culture; B) large scale culture.

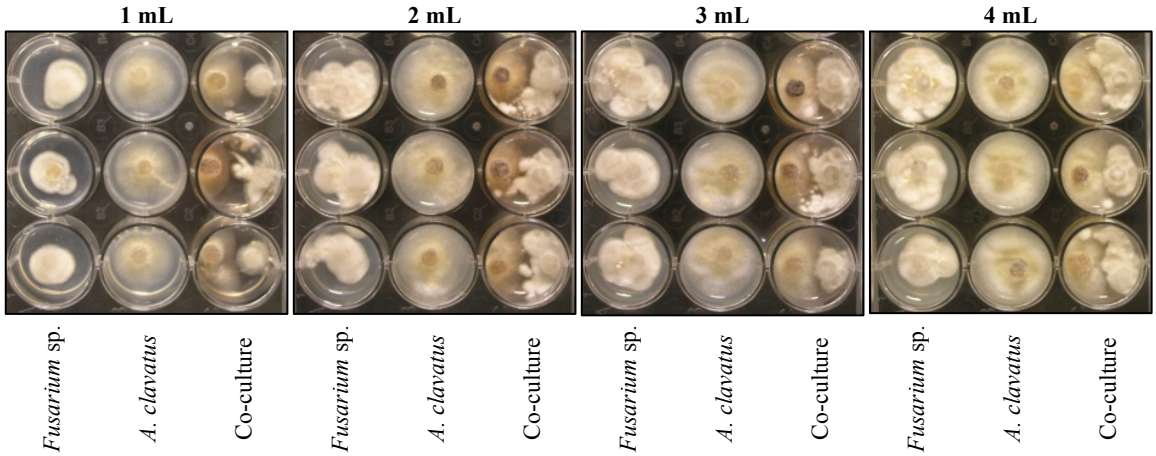

Fig. S2. Pictures of the fungal colony in the 2 cm diameter Petri dish (12-well plates) showing the influence of the culture medium volume on the fungal growth.

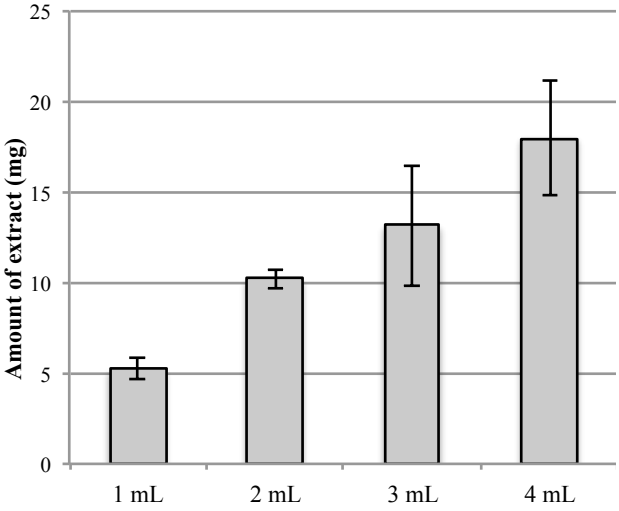

Fig. S3. Amount of fungal co-culture extract obtained depending on the amount of culture medium added to the 2 cm diameter Petri dish (12-well plates).

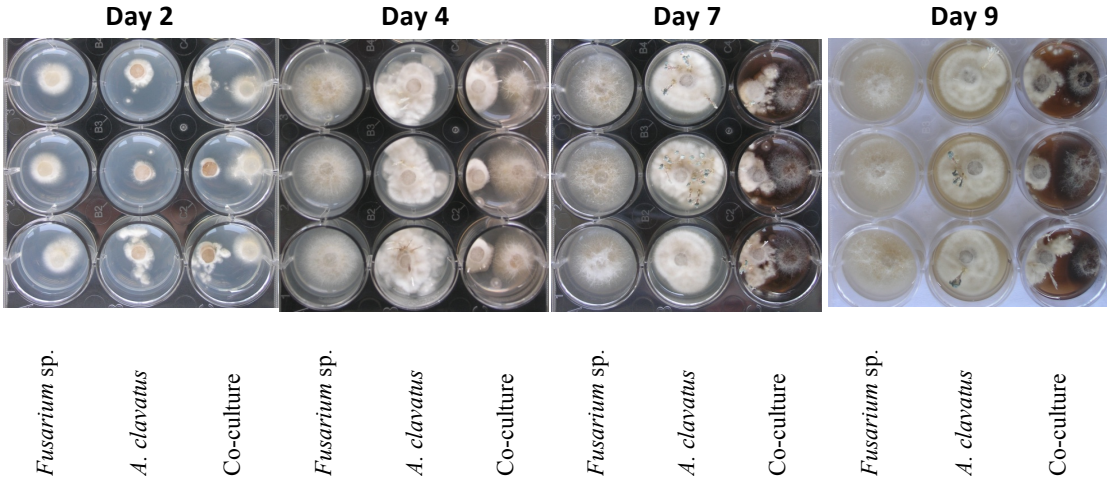

Fig. S4. Morphology of the different colonies at day 2, 4, 7 and 9.

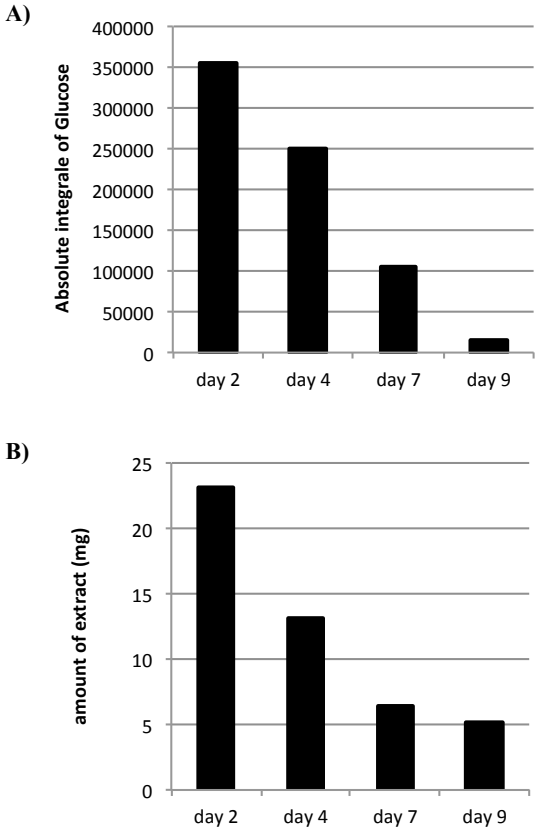

Fig. S5. Evolution of the amount of glucose (α + β) during the co-culture fungal growth based on ^{1}H NMR integration of their anomeric proton (δ_H 5.2 and 4.5 ppm) (A) in regard to the evolution of the amount of extract obtained (B).

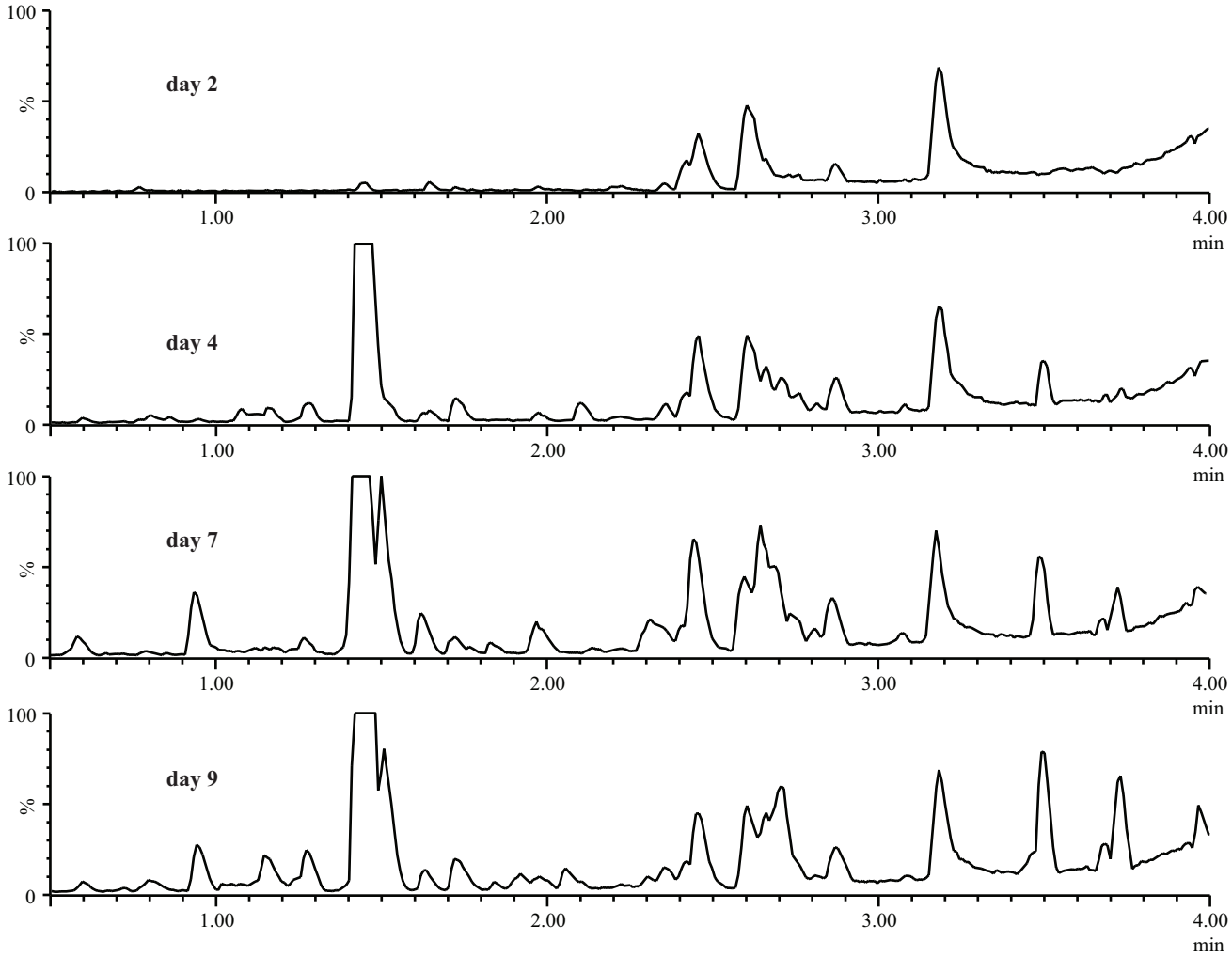

Fig. 6 UHPLC-TOFMS profiles of the co-culture extracts at the four time points analyzed (2, 4, 7 and 9 days).

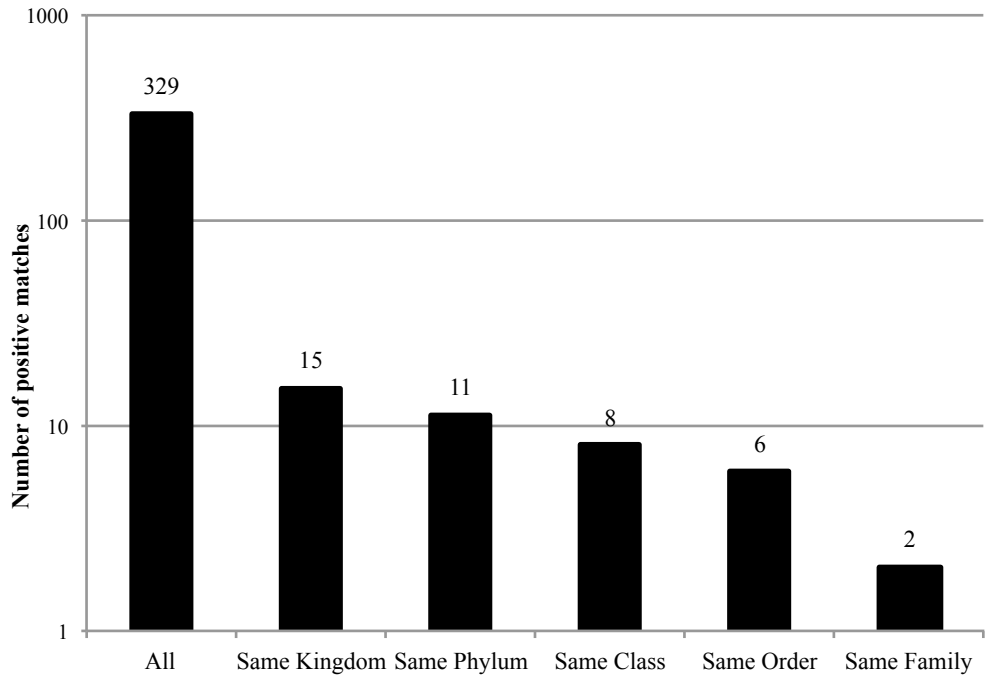

Fig. S7. Number of positive matches (mean values of all the highlighted metabolites in that study) in the Dictionary of Natural Product (*Dictionary of Natural Products on DVD* 2012 Vol. 21:2, CRC Press, Taylor & Francis Group) based on HR-MS data (15 ppm accuracy) and limited to biological source similarities (according to Catalogue of Life, Species2000, http://www.catalogueoflife.org).

Fig. S8. Large-scale fungal co-culture between *A. clavatus* (on the sides) and *Fusarium* sp. obtained after 24 days of growth in 15 cm Petri dishes.

Table S1. List of the *de novo* induced biomarkers with their putative identification based on Dictionary of Natural Product (*Dictionary of Natural Products on DVD* 2012 Vol. 21:2, CRC Press, Taylor & Francis Group).

RT (min)	*m/z* (Da)		Day of *de novo* induction	Putative identification based on the Dictionary of Natural Products
	NI	PI		
Produced by *Fusarium* sp.				
0.60	175.060		7	-
1.28	151.039		9	Gibepyrone F[b]
1.46		289.069	4, 7	TM[b]
1.85		286.070	7, 9	Bostrycoidin[b] $[M+H]^+$
Produced by *A. clavatus*				
0.77		194.153	9	-
2.55		444.162	9	Fumiquinazoline C or D[a] $[M+H]^+$
2.69	476.277		2	-
3.15		585.391	7	-
3.18	588.353		4	-
Unknown Producer				
0.77	387.038		4	TM[a,b]
0.80	525.071		9	-
1.03	445.114		9	-
1.15	581.131		9	-
1.44	205.087		7, 9	TM[a,b]
1.44		481.032	9	-
1.44	427.103		7, 9	(S)-Mitorubrin[a] $[M+HCO_2]^-$
1.46	305.066		7	TM[a,b]
2.55		265.178	9	Brefeldin A[a] $[M+H]^+$

[a]: closely related to *A. clavatus;* [b]: closely related to *Fusarium sp.*; TM: Too many possibilities reported to putatively identify the compound

Table S2. Steps and parameters used during the automatic peak piking procedure by MZmine 2.

Steps	Parameters	Value	
		NI	PI
1) Raw data methods -> Filtering -> Dataset Filtering			
Filter	Crop filter		
	Retention time (min)	0.4 to 4.5	0.4 to 4.5
2) Raw data methods -> Peak detection -> Mass detection			
Mass detector	Centroid		
	Noise Level	30	30
MS level		1	1
3) Raw data methods -> Peak detection -> Chromatogram builder			
Min time span (min)		0.03	0.03
Min height		30	30
m/z tolerance (ppm)		5	5
4) Peak list methods -> Peak detection -> Chromatogram deconvolution			
Min peak height		30	30
Peak duration range (min)		0.03 - 10	0.03 - 10
Baseline level		10	10
5) Peak list methods -> Isotopes -> Isotopic peaks grouper			
m/z tolerance (ppm)		5	5
Retention time tolerance (min)		0.03	0.03
Maximum charge		3	3
Representative isotope		Most intense	
6) Peak list methods -> Alignment -> Join aligner			
m/z tolerance (ppm)		15	15
Weight for *m/z*		10	10
Retention time tolerance (min)		0.2	0.2
Weight for RT		10	10
7) Removal of the peaks from blanks and Agar samples from the generated matrix			
8) Peak List methods -> Gap filling -> Peak finder			
Intensity tolerance (%)		100	100
m/z tolerance (ppm)		15	15
Retention time tolerance (min)		0.2	0.2