

Supplementary Material

Investigating the Transformation Products of Selected Antibiotics and 17 α -Ethinylestradiol under Three In Vitro Biotransformation Models for Anticipating Their Relevance in Bioaugmented Constructed Wetland

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Number of Tables: 5

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Table S1. Identification of SMX biotransformation products

Compound	RT (min)	m/z (error)	Molecular formula	Characteristic fragments	Molecular structure	Identification confidence (structural modification)	Laccase and peroxidase	Trichoderma	Tobacco		Horseradish	
									tissue	media	tissue	media
SMX	6.3	254.0601 (0.70 ppm)	C ₁₀ H ₁₁ N ₃ O ₃ S	188.0811 156.0106 108.0443		1						
SMX416	4.9	416.1117 (-0.51 ppm)	C ₁₆ H ₂₁ N ₃ O ₈ S	336.0714 254.0586 216.1229		2b (+ glucose - H ₂ O)		x	x	x	x	x
SMX578	2.6	578.1640 (0.31 ppm)	C ₂₂ H ₃₁ N ₃ O ₁₃ S	416.1078 336.0712 254.0580		2b (+ 2 glucose - 2(H ₂ O))					x	x
SMX296	6.4	296.0703 (1.20 ppm)	C ₁₂ H ₁₃ N ₃ O ₄ S	198.0215 134.0598		2a (N-acetylation)	x	x	x		x	x

Table S2. Identification of TMP biotransformation products

Compound	RT (min)	m/z (error)	Molecular formula	Characteristic fragments	Molecular structure	Identification confidence (structural modification)	Laccase and peroxidase	Trichoderma	Tobacco		Horseradish	
									tissue	media	tissue	media
TMP	4.6	291.1452 (1.54 ppm)	C ₁₄ H ₁₈ N ₄ O ₃	275.1143 261.0975 230.1153 123.0659		1						
TMP305	5.3	305.1250 (0.55 ppm)	C ₁₄ H ₁₆ N ₄ O ₄	275.0755 244.0936 137.0459		2a (+ O - 2H)	x	x	x	x	x	X
TMP307	3.7	307.1406 (-1.74 ppm)	C ₁₄ H ₁₈ N ₄ O ₄	289.1299 274.1059 259.0820		2a (+ OH)	Only peroxidase			x	x	x

TMP277	6.0	277.1312 (0.75 ppm)	C ₁₃ H ₁₆ N ₄ O 3	261.0994 247.0814 123.0660		3 (- CH ₃)			X	x		x
TMP261	4.4	261.1352 (0.55 ppm)	C ₁₃ H ₁₆ N ₄ O 2	245.1032 136.0756 123.0667		3 (- CH ₃ O)		x		x		x
TMP325	4.1	325.1506 (0.02 ppm)	C ₁₄ H ₂₀ N ₄ O 5	181.0858 143.0566		3 (+ 2OH)				x		x
TMP321	5.2	321.1545 (-1.23 ppm)	C ₁₅ H ₂₀ N ₄ O 4	289.1289 274.1054 259.0821		3 (+ CH ₃ + OH)			x	x	x	x
TMP453	4.2	453.1964 (-0.85 ppm)	C ₂₀ H ₂₈ N ₄ O 8	435.1856 357.1559 291.1448		2b (+ glucose - H ₂ O)			x		x	x

Table S3. Identification of OFL biotransformation products

Compound	RT (min)	m/z (error)	Molecular formula	Characteristic fragments	Molecular structure	Identification confidence (structural modification)	Laccase and peroxidase	Trichoderma	Tobacco		Horseradish	
									tissue	media	tissue	media
OFL	4.8	362.1505 (-1.43 ppm)	C ₁₈ H ₂₀ N ₃ O 4	318.1608 261.1030		1						
OFL378	5.2	378.1458 (-0.51 ppm)	C ₁₈ H ₂₀ N ₃ O 5	361.1427 317.1528 247.0874		2b (+ OH)	x	x	x	x	x	X
OFL394	4.5	394.1399 (-2.54 ppm)	C ₁₈ H ₂₀ N ₃ O 6	376.1303 231.0765 176.0704		2b (+ 2OH)	x					x

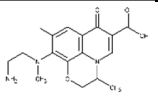
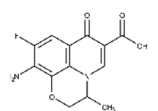
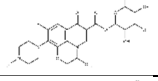
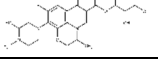
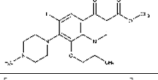
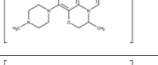
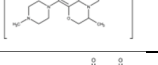
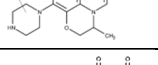
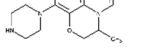
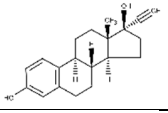
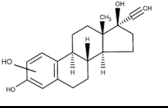
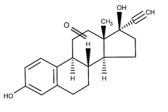
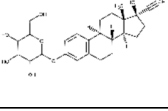
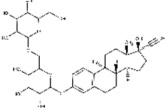
OFL336	4.7	336.1340 (-4.10 ppm)	C16H18FN3O 4	316.1275 279.0769 261.1029		2b (- C2H4)	x	x	x	x	x	x
OFL279	6.3	279.0777 (0.6 ppm)	C13H11FN2O 4	261.0675 238.0384 191.0248		2b (- methyl pyrimidine ring)	x	x	x	x	x	X
OFL-524	1.6	524.2033 (-1.09 ppm)	C24H30FN3O 9	362.1501 318.1606 233.1492		2b (+ glucose - H2O)			x		x	
OFL540	4.9	540.1973 (-2.77 ppm)	C24H30FN3O 10	363.1534 261.1033		3 (+ glucose - H2O + OH)						
OFL376-a	4.1	376.1660 (-0.64 ppm)	C19H22FN3O 4	362.1507 319.1087 305.0930		2a (+ CH3)			x		x	
OFL376-b	1.5	376.1299 (-1.26 ppm)	C18H18FN3O 5	358.1215 213.1019 188.0702		3 (+O - 2H)			x		x	
OFL376-c	6.5	376.1292 (0.70 ppm)	C18H18FN3O 5	358.1215 213.1019 188.0702		3 (+O - 2H)	x					x
OFL364	4.4	364.1293 (1.99 ppm)	C17H18FN3O 5	346.1191 279.0794 229.0747		3 (+OH - CH3)	Only laccase	x				x
OFL348	4.6	348.1368 (3.9 ppm)	C17H18FN3O 4	325.0184 304.1434 261.1033		2a (- CH3)	Only laccase	x	x	x	x	X

Table S4. Identification of EE2 biotransformation products

Compound	RT	m/z							Tobacco	Horseradish
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	(min)	(error)	Molecular formula	Characteristic fragments	molecular structure	Identification confidence (structural modification)	Laccase and peroxidase	Trichoderma	tissue	media	tissue	media
EE2	8.7	295.1703 (2.46 ppm)	C ₂₀ H ₂₄ O ₂	277.1598 269.1556 159.0815 145.0659		1					x	
EE2-311-a	5.5	311.1661 (2.67 ppm)	C ₂₀ H ₂₄ O ₃	293.1539 209.0973 195.0816 145.0659		3 (+ OH)			x			
EE2-311-b	6.6	311.1661 (2.67 ppm)	C ₂₀ H ₂₄ O ₃	293.1539 209.0973 195.0816 161.0609 145.0659		3 (+ OH)	x		x	x		
EE2-311-c	6.8	311.1661 (2.67 ppm)	C ₂₀ H ₂₄ O ₃	293.1539 209.0973 195.0816		3 (+ OH)	Only laccase	x		x		
EE2-311-d	7.2	311.1661 (2.67 ppm)	C ₂₀ H ₂₄ O ₃	293.1539 209.0975 195.0816 175.0768 145.0659		3 (+ OH)	x		x	x	x	x
EE2-311-e	7.9	311.1661 (2.67 ppm)	C ₂₀ H ₂₄ O ₃	293.1541 209.0973 195.08116 175.0768 161.0609		3 (+ OH)		x	x		x	x
EE2-309	7.5	309.1498 (0.52 ppm)	C ₂₀ H ₂₂ O ₃	291.1966 171.1035 137.0973 119.0875		3 (+O - 2H)		x	x	x	x	x
EE2-457	6.9	457.2231 (-0.17 ppm)	C ₂₆ H ₃₄ O ₇	337.1798 295.1727 267.1393 133.0247		2b (+ glucose - H ₂ O)			x	x		
EE2-619	6.1	619.2782 (3.55 ppm)	C ₃₂ H ₄₄ O ₁₂	457.2252 323.0996 295.1724		2b (+ 2 glucose - 2H ₂ O)			x	x		

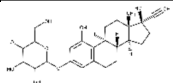
EE2-473	6.6	473.2175 (2.66 ppm)	C ₂₆ H ₃₄ O ₈	311.1654 171.1035 128.0355		3 (+ OH + glucose - H ₂ O)			x	x		
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Table S5: Average EPs removal rates (in %) in tobacco and horseradish HR (Nt-HR and AR-HR) and *Trichoderma asperellum* strain T34 (*Ta* T34) cultures after 4 d of incubation (n = 3).

	<i>AR</i> -HR	<i>Nt</i> -HR	<i>Ta</i> T34
SMX	98	99	95
TMP	37	60	60
OFL	-	77	80
EE2	100	99.8	98

Figure S1. POD activity determined in tissue and culture medium of *A. rusticana* (A and B) and *N. tabacum* HR (C and D). HRCs of 15 d of growth on MS medium and then were treated with 1 ppm (1000 ng/mL) of a mixture of emerging pollutants (SMX, TMP, OFL and EE2). Light and dark grey columns indicate untreated and treated HR cultures with the emerging pollutants, respectively.

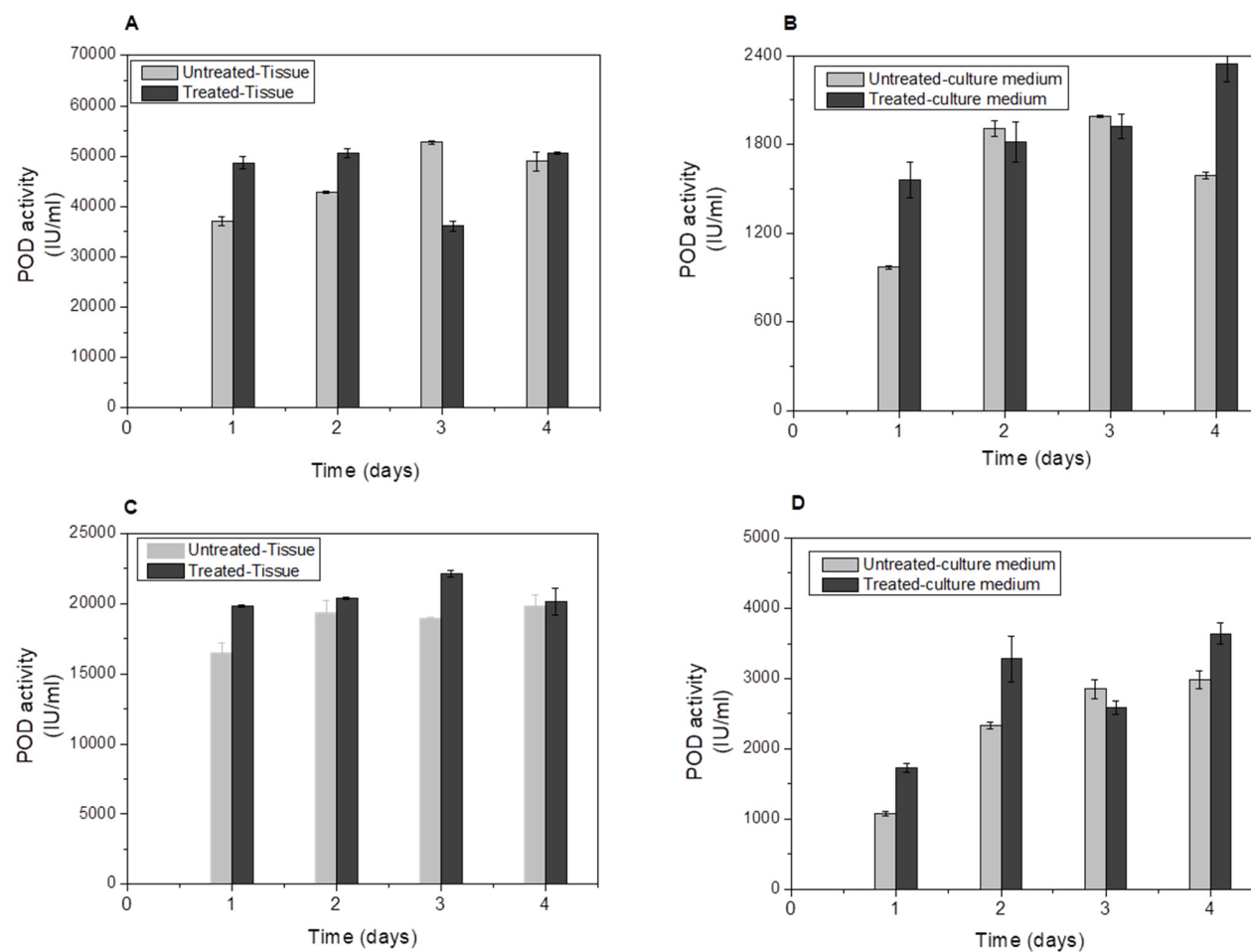


Figure S2. Transformation products detected in the culture medium of *T. asperellum* (A, B, C and D) after 4 d of SMX, TMP, OFL and EE2 treatment (1 ppm).

