Supporting Information

Combination of isotope labeling and molecular networking of tandem mass spectrometry data to reveal 69 unknown metabolites produced by *Penicillium nordicum*

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Fungal cultures on unlabeled and labeled media

The wheat variety Caphorn of *Triticum aestivum* was uniformly enriched with stable isotopes according to a previously developed method[1]. The wheat was grown over 6 months in hermetically sealed chambers with ^{13}C-labeled carbon dioxide ($^{13}CO_2$) with or without ^{15}N-labeled nutrients ($^{15}NO_3^-$ and $^{15}NH_4^+$ ions) to generate two different enrichment patterns. The first wheat (referred to as ^{13}C wheat) was grown with only $^{13}CO_2$ and was enriched with nearly 100% ^{13}C. The second (referred to as $^{13}C/^{15}N$ wheat) was grown with 50% $^{13}CO_2$ and 50% $^{12}CO_2$ as the carbon source and with 100% ^{15}N-labeled nutrients as the nitrogen sources. A Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific) was used to measure the isotopic enrichment: 96.80% ± 0.15% for ^{13}C wheat and 53.40% ± 0.30% ^{13}C and 96.80% ± 0.07% ^{15}N for $^{13}C/^{15}N$ wheat.

Natural and labeled wheat grains were soaked in water and autoclaved. Before inoculation, an HC2-AW sensor (Rotronik AG, Basserdorf, Switzerland) was used to measure the water activity (a_w) of the grains. Thirty grams of each type of grain was then placed in a thin layer in sterile 140 mm Petri dishes (Sarstedt, Marnay, France), and the water activity was adjusted with sterile water to reach a a_w of 0.98. The first dish contained ^{13}C wheat, the second dish received $^{13}C/^{15}N$ wheat, and two other dishes were filled with ^{12}C wheat. The fungus was cultured in the first three plates by inoculation with 0.250 mL of a spore solution (at 10^5 spores/mL). The second ^{12}C wheat Petri plate was not inoculated but was stored under the same conditions as the other Petri dishes to serve as a control. The fungal cultures on natural and labeled wheats as well as the control were stored in the dark without shaking for one month at 25 °C.

[1]Pean, M.; Boiry, S.; Ferrandi, J.-C.; Gibiat, F.; Puel, O.; Delaforge, M. Production and Use of Mycotoxins Uniformly Enriched with Stable Isotopes for Their Dosage in Biological Samples: (1) Production of Uniformly Enriched Biomass. J. Label. Compd. Radiopharm. 2007, 50 (5–6), 563–564.

Table S1.

HCD and CID product ion spectra of secondary metabolites detected in *P. nordicum* after culture on wheat grains. Numbers in brackets refer to the number attached to each metabolite throughout the article. [a] Elemental compositions were calculated by comparison of the *m/z* ratios obtained from the culture of *P. nordicum* in ^{12}C wheat, 97% ^{13}C wheat and 53% ^{13}C / 96% ^{15}N wheat. Product ions in bold: found in both experimental spectra and *in silico* spectra generated with CFM-ID.

	Molecular formula[a]	Delta ppm	Parent ion	^{12}C *m/z*	R_T (min)	Activation mode (NCE%)	*m/z* of major product ions (relative abundance in HCD mode - relative abundance in CID mode)
	C_5H_9NO	-1.9	$[M+H]^+$	100.0755	2.9	HCD(35) CID(25)	100.0753(100-100)
(6)	**$C_6H_{10}O_4$**	-1.9	$[M+H]^+$	147.0649	3.1	HCD(20) CID(25)	147.0640(5-10) 129.0542(100-100) 117.0543(30-72) 97.0280(15-5) 85.0280(15-6) 99.0437(8-2) 87.0437(8-2) 75.0437(6-2) 71.0488(5-0) 55.0017(5-0) 115.0387(3-1)
	$C_{10}H_{13}NO_2$	-1.6	$[M+H]^+$	180.1016	3.9	HCD(20) CID(25)	180.1016(100-12) 121.0644(95-0) 138.0909(22-70) 162.0909(0-1)
(10)	**$C_{11}H_{16}O_4$**	-0.6	$[M+H]^+$	213.1120	15.6	HCD(20) CID(25)	**213.1119**(100-45) **195.1012**(1-50) 111.0438(0-100) 167.1062(0-90) 139.0386(0-27) **153.0906**(0-24) **181.0854**(0-20) 125.0229(0-17) 107.0853(0-12) **125.0229**(0-20) **109.0645**(0-10) **99.0439**(0-1) **97.0280**(0-2) **97.0645**(0-2) **95.0488**(0-1)
(9)	$C_{11}H_{16}O_5$	0.2	$[M+H]^+$	229.1071	5.5	HCD(20) CID(15)	229.1071(100-4) 211.0960(95-100) 141.0542(35-35) 125.0229(30-5) 157.0491(10-7) 193.0858(6-3)
	$C_{11}H_{16}O_5$	0.2	$[M+H]^+$	229.1071	6.1	HCD(20) CID(15)	229.1071(70-45) 211.0693(100-100) 141.0544(27-15) 152.0231(25-2) 157.0494 5-2) 193.0856(4-0) 197.0806(0-1)
(7)	**$C_{11}H_{18}O_4$**	-0.3	$[M+H]^+$	215.1277	13.9	HCD(20) CID(15)	**215.1276**(27-40) **197.1170**(100-100) 153.1272(15-2) **137.0958**(7-1) **171.1376**(2-1) **169.1220**(4-1) **151.1115**(4-1) **183.1013**(3-2) 119.0853(2-0) **155.1063**(2-1) 165.0907(2-0) **179.1066** (2-1)
(8)	**$C_{11}H_{18}O_5$**	-0.4	$[M+H]^+$	231.1226	5.7	HCD(20) CID(25)	**231.1227**(100-42) 181.0857(70-100) **127.0387**(31-70) **213.1119**(25-25) **195.1013**(25-25) 81.0332(32-21) 155.1064(19-40) 139.0750(19-19) 141.0543(14-65) 151.1115(13-6) **199.0662**(6-22) **171.1012**(7-10) **71.0488**(4-1)
	$C_{12}H_9N_3O_2$	-0.8	$[M-H]^-$	226.0620	15.2	HCD(50) CID(25)	226.0620(100-22) 196.0701(12-100) 145.0413(9-16) 183.0569(0-72) 199.0515(0-20) 198.0682(0-15)
	$C_{12}H_9N_3O_2$	-0.8	$[M-H]^-$	226.0620	12.7	HCD(50) CID(25)	226.0620(100-23) 183.0566(7-100) 196.0702(0-47) 145.0412(0-40) 199.0516(0-25)
	$C_{12}H_9N_3O_2$	-0.8	$[M-H]^-$	226.0620	18.0	HCD(50) CID(25)	226.0620(100-10) 196.0702(20-100) 183.0569(0-42) 145.0413(0-20) 199.0513(0-10) 195.0670(0-10) 198.0682(0-6)
	$C_{13}H_{17}NO$	-0.6	$[M-H]^-$	202.1236	12.8	HCD(50) CID(25)	202.1236(100-20) 131.0540(4-100) 103.0559(3-55) 158.0616(0-12) 175.1133(0-8) 124.0772(0-7)
	$C_{14}H_{26}O_5$	-1.4	$[M+H]^+$	275.1849	18.1	HCD(20) CID(25)	275.1849(15-2) 109.1009(100-3) 257.1744(55-100) 221.1532(60-4) 239.1639(42-7) 127.1114(40-5) 161.1321(22-0) 203.1425(15-0) 67.0539(15-0) 175.1476(7-0) 95.0852(4-0)
	$C_{15}H_{24}O_2$	-0.8	$[M+H]^+$	237.1847	17.2	HCD(20) CID(15)	237.1847(72-7) 219.1740(100-100) 205.0854(5-5) 163.1113(0-6) 173.0591(5-3) 161.1322(0-3) 191.1167(2-2) 201.1634(0-2) 179.1062(0-1)
	$C_{15}H_{24}O_2$	-0.8	$[M+H]^+$	237.1847	22.1	HCD(20) CID(15)	237.1847(100-75) 219.1739(10-100) 177.1633(3-30) 109.1009(5-25) 121.1007(0-21) 201.1633(0-21) 95.0852(6-19) 181.1216(0-19) 137.1320(0-17) 191.1788(0-12) 167.1062(0-11)
	$C_{15}H_{24}O_2$	-0.8	$[M+H]^+$	237.1847	23.1	HCD(20) CID(15)	237.1847(100-75) 219.1738(12-100) 177.1633(0-32) 109.1009(5-26) 121.1008(0-24) 95.0851(5-22) 137.1320(0-19) 181.1217(0-19) 201.1634(0-19) 191.1789(0-15) 167.1062(0-13)
	$C_{15}H_{24}O_3$	-0.4	$[M+H]^+$	253.1797	9.7	HCD(20) CID(15)	253.1794(55-3) 235.1689(100-100) 217.1583(12-6) 193.1217(0-3) 177.1269(5-3) 151.0749(8-1) 189.1633(3-1)
	$C_{15}H_{24}O_3$	-0.4	$[M+H]^+$	253.1797	22.1	HCD(20) CID(15)	253.1794(100-50) 235.1689(52-100) 207.1739(35-52) 179.1062(15-19) 137.0957(7-10) 189.1633(7-15) 217.1583(5-15) 165.0906(5-5) 175.1476(5-10) 191.1790(0-2)
		-1.0	$[M-H]^-$	251.1650		HCD(50) CID(15)	251.1650(100-100) 207.1758(0-25) 137.0975(0-12) 236.0689(0-5)
	$C_{15}H_{24}O_3$	-1.0	$[M-H]^-$	251.1650	35.5	HCD(50) CID(25)	251.1650(100-3) 205.1602(0-100) 233.1553(0-50) 223.1706(0-5)
	$C_{15}H_{24}O_3$	-0.4	$[M+H]^+$	253.1797	23.2	HCD(20) CID(15)	253.1795(100-2) 235.0853(50-100) 207.1735(35-62) 179.1060(29-31) 217.1584(0-14) 189.1634(0-16) 175.1477(0-11) 137.0958(0-7) 165.0906(0-4)
		-1.0	$[M-H]^-$	251.1650		HCD(50) CID(15)	251.165(100-100) 207.1758(0-30) 137.0675(0-7) 236.0695(0-4)
	$C_{15}H_{26}O_2$	-1.2	$[M-H]^-$	237.1857	40.4	HCD(50) CID(25)	237.1857(100-3) 168.1148(15-100) 196.1096(0-17) 210.1252(0-14)

(5)	**$C_{16}H_{11}N_3O_2$**	-0.7	$[M+H]^+$	278.0922	9.9	HCD(20) CID(15)	278.0922(100-40) 249.0657(0-100) 235.0865(0-12) 250.0970(0-4) 146.0234(0-4) 233.0707(0-2) 175.0498(0-2) 132.0441(0-1) 105.0333(0-1)
	$C_{16}H_{11}N_3O_2$	-0.7	$[M+H]^+$	278.0922	15.1	HCD(35) CID(25)	278.0922(100-100) 250.0972(1-60) 233.0706(0-30) 249.0656(1-14) 248.0815(0-13) 235.0863(0-10) 132.0440(0-6) 260.0813(0-3) 222.1022(0-1)
	$C_{16}H_{14}O_3$	-3.2	$[M-H]^-$	253.0862	11.5	HCD(50) CID(25)	253.0862(100-3) 159.0457(0-100) 235.0770(0-82)
	$C_{17}H_{10}O_5$	-2.0	$[M+H]^+$	295.0595	11.2	CID(25)	295.0596(60) 238.0620(100) 250.0858(25) 267.0650(20) 250.0585(17) 223.0748(15) 278.0804(16) 205.0643(13) 251.0697(11) 239.0696(10) 120.0804(6)
		-1.8	$[M-H]^-$	293.0450		HCD(50) CID(25)	293.0450(100-2) 249.0561(0-100)
	$C_{17}H_{14}N_2O_3$	-2.9	$[M-H]^-$	293.0923	11.3	CID(25)	293.0923(3) 249.0562(100)
	$C_{17}H_{26}O_4$	-2.1	$[M-2H+Na]^-$	315.1571	38.6	CID(25)	315.1571(2) 211.0365(100) 210.0330(80) 239.1446(75) 167.0273(20) 298.0723(19) 168.0306(19) 255.1970(5) 271.1702(5)
	$C_{17}H_{34}O_5$	-4.2	$[M-H]^-$	317.2320	39.1	HCD(50) CID(25)	317.2320(100-2) 257.2129(13-100) 299.2222(0-25) 255.2332(0-15) 213.0336(0-4) 212.0301(0-3) 115.0769(0-4)
	$C_{17}H_{34}O_5$	-4.2	$[M-H]^-$	317.2320	39.5	HCD(50) CID(25)	317.2330(100-2) 257.2126(9-100) 299.2219(0-12) 255.233(0-7)
	$C_{17}H_{34}O_5$	-4.2	$[M-H]^-$	317.2320	41.2	HCD(50) CID(25)	317.2310(100-2) 257.2129(15-100) 299.223(0-23) 255.2333(0-16) 115.0770(0-5) 195.1757(0-4)
	$C_{17}H_{34}O_5$	-4.2	$[M-H]^-$	317.2320	38.1	HCD(50) CID(25)	317.2320(100-3) 257.2129(23-100) 299.2225(0-18) 273.2591(0-20) 255.2333(0-15) 115.0769(0-5) 219.2119(0-4) 195.1758(0-3)
	$C_{17}H_{34}O_5$	-4.2	$[M-H]^-$	317.2320	41.0	HCD(50) CID(25)	317.2320(100-4) 257.2128(14-100) 299.2236(2-22) 255.2333(0-16) 115.0769(0-5) 195.1756(0-3)
(4)	**$C_{18}H_{20}N_4O_3$**	-0.6	$[M+H]^+$	341.1606	15.2	HCD(20) CID(25)	341.1601(60-3) **324.1337**(100-100) **296.1388**(10-2) 151.1114(3-0) 195.1012(1-0)
		-0.6	$[M-H]^-$	339.1458		HCD(50) CID(25)	339.1458(100-2) 268.1097(50-100) 183.0124(0-10) 284.0918(0-4) 324.0638(0-5)
	$C_{18}H_{20}N_4O_3$	-1.3	$[M+H]^+$	341.1606	20.1	HCD(20) CID(25)	341.1605(57-3) 324.1338(100-100) 296.1389(7-2)
(3)	**$C_{18}H_{22}N_4O_3$**	-1.0	$[M+H]^+$	343.1761	12.7	HCD(20) CID(25)	343.1758(45-2) 326.1496(100-100) 298.1546(5-1)
	$C_{18}H_{22}N_4O_3$	-1.0	$[M+H]^+$	343.1761	17.8	HCD(20) CID(15)	343.1756(45-49) 326.1494(100-100) 298.1544(6-1) 270.1544(3-0)
	$C_{18}H_{22}N_4O_4$	-3.2	$[M+Na]^+$	381.1521	15.3	HCD(35) CID(15)	381.1521(100-45) 280.0687(50-56) 298.0792(30-100) 279.0848(22-44) 364.1255(4-42) 363.1424(12-25) 169.0369(5-0) 281.0526(0-30) 254.0894(0-11) 337.1626(0-5) 261.1226(0-3)
	$C_{18}H_{24}N_2O_2$	-3.5	$[M+H]^+$	301.1900	37.7	HCD(35) CID(25)	301.1908(100-47) 283.1809(5-72) 227.1178(4-100) 258.1358(1-62) 240.1260(0-71) 228.1623(0-45) 255.1860(0-23) 257.2017(0-16) 199.1231(0-13) 256.1938(0-11) 212.1308(0-6)
	$C_{19}H_{37}NO_2$	-0.3	$[M+H]^+$	312.2896	31.8	HCD(20) CID(15)	312.2890(6-1) 294.2788(100-100) 86.0597(6-0) 276.2685(4-3) 259.2418(4-4) 277.2528(2-4) 264.2682(2-0)
(1)	**$C_{20}H_{18}NO_6Cl$**	-0.3	$[M+H]^+$	404.0894	33.2	HCD(20) CID(15)	404.0891(31-70) 358.0836(100-100) 239.0102(65-3) 341.0571(12-2) 257.0208(11-0) 120.0804(11-0) 386.0784(4-25)
		0.02	$[M-H]^-$	402.0750		HCD(50) CID(25)	402.0750(37-2) 358.0856(100-100) 211.0171(10-1) 254.0229(6-2) 322.1088(4-3) 270.0696(4-4) 340.0750(3-3) 239.0122(3-0) 296.0852(2-1)
(2)	**$C_{20}H_{19}NO_6$**	-0.5	$[M+H]^+$	370.1283	26.5	HCD(20) CID(25)	370.1283(32-2) 205.0491(100-17) 324.1225(37-100) 223.0597(7-0) 352.1171(7-20) 307.0959(7-5) 120.0804(7-5)
		-0.4	$[M-H]^-$	368.1138		HCD(50) CID(25)	368.1138(45-2) 324.1247(100-100) 220.0621(8-4) 280.1347(3-2) 164.0722(3-0) 306.1141(0-1)
	$C_{20}H_{30}O_4$	-2.7	$[M-H]^-$	333.2062	39.4	HCD(50) CID(25)	333.2062(100-2) 163.0768(0-100) 289.2175(0-93) 315.1962(0-40) 235.1344(0-25) 273.2070(0-22) 292.1840(0-16) 318.1993(0-14) 145.0661(0-7)
	$C_{21}H_{30}N_4O_4$	-2.8	$[M-H]^-$	401.2183	35.4	HCD(50) CID(25)	401.2183(100-2) 262.1561(0-100) 172.1093(0-67) 357.1342(0-47) 245.1299(0-45) 228.1028(0-16) 203.0828(0-15) 383.2090(0-9) 137.0723(0-9) 236.1407(0-9) 214.1552(0-4)
	$C_{21}H_{30}N_4O_4$	-2.8	$[M-H]^-$	401.2183	25.8	HCD(50) CID(25)	401.2183(100-3) 172.1094(12-70) 262.1562(0-100) 245.1300(0-45) 383.2092(0-21) 203.0828(0-22) 228.1033(0-22) 214.1562(0-18) 137.0723(0-12) 283.1817(0-12) 236.1406(0-12)
	$C_{21}H_{32}N_2O_2$	-1.3	$[M+H]^+$	345.2532	27.0	HCD(35) CID(15)	345.2532(100-100) 245.2009(30-75) 244.1931(17-37) 274.1670(10-30) 174.1147(7-23) 330.2292(2-5) 231.1851(0-4) 285.2316(0-1) 328.1549(0-1) 327.1943(0-2)
	$C_{21}H_{32}N_2O_2$	-1.3	$[M+H]^+$	345.2532	23.5	HCD(35) CID(15)	345.2532(100-100) 245.2009(20-25) 244.1932(15-13) 274.1673(8-12) 174.1149(5-9) 330.2298(3-3) 149.1071(1-0) 231.1852(1-1) 327.2423(0-1) 285.2319(0-1)
	$C_{21}H_{32}N_4O_5$	-3.3	$[M-H]^-$	419.2286	17.1	CID(25)	419.2286(3) 186.0563(100) 259.1455(40) 389.1575(25)
	$C_{21}H_{36}O_3$	-4.2	$[M+H]^+$	337.2723	37.1	HCD(20) CID(25)	337.2742(100-50) 263.2372(6-100) 161.1326(0-75) 175.1481(0-72) 147.1168(0-69) 245.2267(3-67) 133.1012(0-63) 121.1011(0-59) 163.1482(0-59) 149.1325(0-55) 337.2742(0-51)

	$C_{22}H_{28}N_4O_5$	-2.9	[M-2H+Na]$^-$	449.1793	25.4	CID(25)	449.1808(2) 202.0599(100) 282.1249(73)
	$C_{23}H_{26}N_2O_4$	-1.0	[M-2H+Na]$^-$	415.1635	17.9	CID(25)	415.1640(2) 268.0957(100) 246.1139(65)
	$C_{25}H_{32}N_4O_5$	-3.2	[M+H]$^+$	469.2430	21.2	HCD(20) CID(15)	469.2430(12-2) 332.1961(100-100) 205.0966(40-10) 148.0752(8-1) 177.1016(5-0) 265.1540(4-1) 100.1116(1-0)
	$C_{25}H_{32}N_4O_5$	-3.2	[M+H]$^+$	469.2430	24.1	HCD(20) CID(15)	469.2430(23-2) 332.1961(100-100) 205.0966(55-16) 148.0752(15-2) 177.1016(6-2) 265.1540(5-2) 100.1117(1-0) 441.1897(2-0)
	$C_{25}H_{40}N_4O_5$	-2.4	[M+H]$^+$	477.3060	16.9	HCD(20) CID(15)	477.3060(30-2) 265.1550(100-65) 213.1592(45-16) 185.1642(36-6) 312.2272(30-26) 459.2964(0-10) 431.3003(0-8) 166.0858(5-3) 72.0804(5-0) 86.0960(5-0) 378.2373(1-4) 120.0804(2-0) 267.2079(2-4)
	$C_{26}H_{42}O_7$	-4.9	[M+H]$^+$	467.2980	30.5	HCD(20) CID (15)	467.2999(12-2) 305.2472(100-100) 109.0646(25-0) 323.2574(16-20) 287.2370(12-12) 209.1896(8-4) 149.1323(7-0) 449.2884(0-16) 183.1013(0-5) 269.2263(0-4) 365.2682(0-18)
	$C_{28}H_{36}N_4O_6$	-4.8	[M+H]$^+$	525.2682	25.8	HCD(20) CID(15)	525.2680(15-2) 346.2115(100-26) 426.2016(80-80) 247.1436(60-5) 497.2742(0-100) 327.1331(55-6) 299.1383(55-0) 378.2013(40-7) 152.0701(25-0) 279.1334(22-2) 120.0803(22-0)
		-3.6	[M-H]$^-$	523.2543		HCD(50) CID(15)	523.2543(20-100) 401.2204(100-8)
(15)	**$C_{28}H_{38}N_4O_5$**	-1.7	[M+H]$^+$	511.2906	18.0	HCD(20) CID(15)	511.2906(10-100) 265.1542(100-85) 219.1488(35-10) 247.1437(25-13) 120.0804(16-0) 394.2120(8-22) 412.2228(7-4) 295.1437(3-0) 349.1905(3-6) 493.2799(0-6) 465.2876(0-8) 72.0808(2-0)
		0.1	[M-H]$^-$	509.2770		HCD(50) CID(15)	509.2770(100-100) 276.1033(93-0) 116.0722(83-0) 115.0882(32-0) 164.0722(25-0) 202.1242(15-0) 263.1404(18-0) 262.1565(13-0) 246.1140(8-0) 146.0616(12-0) 465.2884(6-0)
(21)	**$C_{28}H_{38}N_4O_5$**	-1.7	[M+H]$^+$	511.2906	18.7	HCD(20) CID(15)	511.2906(15-100) 265.1542(100-64) 219.1489(66-15) 247.1437(55-35) 346.2118(35-60) 120.0803(10-0) 72.0804(8-0) 301.1904(7-15) 166.0858(6-4) 493.2799(0-4) 394.2116(0-2)
		0.1	[M-H]$^-$	509.2770		HCD(50) CID(15)	509.2770(73-100) 164.0722(100-0) 465.2881(15-0) 147.0457(16-0) 219.1508(7-0) 263.1406(7-0) 361.2253(4-0) 318.2193(4-0) 262.1565(5-0)
(11)	$C_{28}H_{38}N_4O_6$	-2.6	[M+H]$^+$	527.2850	12.7	HCD(20) CID(15)	527.2853(10-100) 265.1541(100-100) 235.1437(32-9) 263.1387(21-12) 120.0803(11-0) 410.2070(7-30) 428.2172(7-3) 136.0753(5-0) 365.1852(3-8) 219.1487(3-2) 311.1384(3-0) 509.2742(0-6)
		-0.1	[M-H]$^-$	525.2718		HCD(50) CID(15)	525.2718(90-100) 292.0982(100-0) 116.0722(70-0) 278.1515(20-0) 172.1095(15-0) 263.1406(13-0) 248.1088(9-0) 162.0567(7-0) 375.2406(5-0) 142.0666(7-0)
	$C_{28}H_{42}O_5$	-4.0	[M+Na]$^+$	481.2905	43.8	HCD(35) CID(15)	481.2905(100-100) 355.2243(21-55)
	$C_{28}H_{42}O_5$	-4.0	[M+Na]$^+$	481.2905	44.2	HCD(35) CID(15)	481.2905(100-100) 355.2243(12-35)
	$C_{28}H_{42}N_4O_5$	0.4	[M+Na]$^+$	537.3050	24.4	HCD(20) CID(15)	537.3050(25-2) 245.1636(100-38) 273.1587(75-88) 372.2281(40-100)
		3.3	[M-2H+Na]$^-$	535.2920		CID(35)	535.2904(3) 491.2972(100) 448.2603(60) 362.2082(90)
	$C_{28}H_{42}N_4O_5$	0.4	[M+Na]$^+$	537.3050	23.7	HCD(20) CID(15)	537.3050(30-2) 273.1587(80-100) 372.2270(0-60) 245.1636(100-60) 420.2267(2-40) 265.1539(0-15)
		3.3	[M-2H+Na]$^-$	535.2920		CID(35)	535.2904 (2) 276.1028(100) 232.1131 (3) 410.2078 (3)
	$C_{28}H_{42}N_4O_5$	0.4	[M+Na]$^+$	537.3050	23.1	HCD(20) CID(15)	537.3050(20-1) 245.1636(100-62) 273.1587(70-100) 265.1539(25-30) 420.2273(15-70) 438.2379(8-8)146.0960(7-0) 519.2935(0-12) 491.3002(0-10) 120.0803(4-0) 174.1269(0-8)
		3.3	[M-2H+Na]$^-$	535.2920		CID(15)	535.2910(2) 410.2088(3) 276.1028(100) 232.1132(10)
	$C_{29}H_{38}N_4O_6$	-0.7	[M+H]$^+$	539.2860	26.9	HCD(20) CID(15)	539.2860(15-2) 120.0803(100-0) 265.1537(35-20) 422.2057(0-100) 521.2787(0-12) 374.2055(0-5) 351.2137(0-4) 412.2214(0-4)
		-3.8	[M-H]$^-$	537.2698		CID(15)	537.2698(100)
(13)	**$C_{29}H_{40}N_4O_5$**	-4.8	[M+H]$^+$	525.3046	19.5	HCD(20) CID(15)	525.3051(13-12) 265.1541(100-100) 261.1594(32-20) 120.0803(15-0) 412.2226(10-2) 219.1487(11-2) 408.2278(8-22) 233.1658(30-10) 507.2984(0-5) 479.3033(0-3) 363.2077(1-10) 295.1453(3-1) 86.0966(2-0)
(12)	**$C_{29}H_{40}N_4O_5$**	-4.8	[M+H]$^+$	525.3046	20.2	HCD(20) CID(15)	525.3060(7-2) 279.1698(100-100) 219.1487(27-12) 247.1437(24-30) 120.0804(15-0) 394.2118(10-10) 426.2382(7-3) 479.3037(0-4) 349.1926(2-10) 295.1455(1-1) 233.1661(1-5) 72.0804(1-0)
(23)	**$C_{29}H_{40}N_4O_6$**	3.3	[M+H]$^+$	541.3039	15.4	HCD(20) CID(15)	541.3039(7-20) 279.1697(100-100) 235.1436(30-5) 263.1384(20-12) 410.2068(11-30) 120.0803(9-0) 442.2329(6-4) 136.0752(6-0) 365.1851(3-6) 523.2893(0-7) 495.2946(0-4) 311.1380(3-0) 233.1656(1-4)
		-2.7	[M-H]$^-$	539.2860		HCD(50) CID(15)	539.2860(100-100) 292.0982(95-1) 130.0877(90-0)
(14)	**$C_{29}H_{40}N_4O_6$**	3.3	[M+H]$^+$	541.3039	13.8	HCD(20) CID(15)	541.3039(10-2) 265.1541(100-100) 249.1592(37-12) 277.1540(30-23) 120.0803(12-0) 428.2170(10-0) 424.2226(10-32) 523.2889(0-

							10) 379.1998(0-8) 311.1383(4-0) 219.1490(2-3) 495.2946(0-4) 283.1453(2-0) 136.0760(3-0) 86.0966(3-0)
		-2.7	$[M-H]^-$	539.2860		HCD(50) CID(15)	539.2860(70-100) 292.0980(100-2) 116.0724(55-0)
	$C_{29}H_{50}N_2O_3$	4.4	$[M+Na]^+$	497.3736	43.6	HCD(20) CID(15)	497.3736(100-90) 217.1335(25-100) 245.2012(1-5) 174.1152(0-12) 159.0718(0-12) 479.3641(0-9) 439.3324(0-5)
	$C_{29}H_{51}NO_2$	0.3	$[M+H]^+$	446.3994	38.6	HCD(35) CID(25)	446.3994(50-2) 429.3721(100-100) 177.0905(38-16) 163.0749(19-0) 137.0593(9-0) 428.3897(0-10) 428.2668(0-9) 387.3259(0-5) 400.2719(0-4) 411.3629(0-3) 417.3731(0-2)
(20)	$\mathbf{C_{30}H_{37}N_5O_4}$	-3.2	$[M+H]^+$	532.2901	36.4	HCD(20) CID(15)	532.2908(75-2) 433.2228(100-82) 504.2947(35-100) 385.2229(90-19) 334.1541(84-28) 159.0913(75-0) 258.1595(62-0) 286.1544(60-4) 346.2117(53-7) 306.1594(35-0) 120.0804(15-0)
		-4.2	$[M-H]^-$	530.2750		CID(15)	530.2755(100) 401.2197(4)
(22)	$\mathbf{C_{30}H_{37}N_5O_5}$	-4.0	$[M+H]^+$	548.2845	29.4	HCD(20) CID(15)	548.2866(45-3) 385.2227(100-28) 449.2172(70-85) 350.1491(62-25) 286.1544(61-4) 258.1595(55-2) 159.0912(55-0) 362.2067(48-10) 520.2906(27-100) 322.1546(25-0) 503.2634(0-20)
		-2.7	$[M-H]^-$	546.2707		HCD(50) CID(15)	546.2707(35-100) 417.2151(100-0) 311.1732(35-0)
	$C_{30}H_{37}N_5O_6$	-3.9	$[M-H]^-$	562.2649	26.2	HCD(50) CID(15)	562.2662(45-100) 311.1729(100-0) 440.2313(45-0) 297.1569(20-0) 433.2106(20-0) 323.1730(12-0)
(19)	$\mathbf{C_{30}H_{39}N_5O_5}$	-1.6	$[M+H]^+$	550.3015	17.4	HCD(20) CID(15)	550.3015(10-95) 304.1649(100-100) 287.1384(35-62) 159.0913(24-1) 433.2230(7-30) 219.1487(7-1) 451.2333(5-2) 247.1436(5-1) 334.1541(4-2) 388.2039(2-10) 532.2935(0-15) 504.2982(0-2) 259.1454(0-1) 120.0811(2-0)
		-0.6	$[M-H]^-$	548.2875		HCD(50) CID(15)	548.2875(100-100) 116.0722(90-0) 315.1142(50-0) 186.0564(45-0) 164.0722(37-0) 419.2308(22-0) 259.1457(25-0) 202.0991(15-0) 375.2407(10-0) 302.1514(20-0) 351.0327(5-0)
(17)	$\mathbf{C_{30}H_{39}N_5O_5}$	-1.6	$[M+H]^+$	550.3015	19.1	HCD(20) CID(15)	550.3015(25-100) 286.1544(100-80) 258.1594(96-32) 265.1540(65-30) 385.2228(50-90) 241.1330(12-14) 364.2222(7-0) 269.1278(6-1) 199.1436(6-0) 166.0858(6-0) 340.2012(1-12) 72.0803(6-0) 533.2770(1-5) 532.2930(0-2) 213.1392(2-0)
		-0.6	$[M-H]^-$	548.2875		HCD(50) CID(15)	548.2875(52-100) 164.0721(100-0) 202.0990(42-0) 375.2406(17-0) 147.0456(15-0) 301.1675(7-0) 504.2989(5-0) 400.2363(4-0) 263.1404(4-0)
(18)	$\mathbf{C_{30}H_{39}N_5O_6}$	-2.4	$[M+H]^+$	566.2959	12.3	HCD(20) CID(15)	566.2965(7-62) 304.1649(100-100) 287.1384(32-68) 159.0913(23-0) 235.1436(10-0) 449.2173(7-35) 467.2280(6-6) 263.1384(7-2) 404.1964(4-10) 548.2852(0-8) 350.1491(4-1) 520.2904(0-2) 136.0751(2-0) 190.1217(1-0) 259.1454(0-2)
		-0.6	$[M-H]^-$	564.2824		HCD(50) CID(15)	564.2824(100-100) 116.0722(95-0) 202.0514(90-0) 331.1091(45-0) 435.2256(30-0) 119.0507(33-0) 302.1514(20-0) 285.1936(18-0) 278.1513(17-0) 318.1464(15-0)
(16)	$\mathbf{C_{30}H_{39}N_5O_6}$	-2.4	$[M+H]^+$	566.2959	13.2	HCD(20) CID(15)	566.2965(25-25) 286.1544(100-88) 385.2228(57-100) 258.1595(99-35) 281.1489(67-38) 241.1330(15-15) 340.2009(0-12) 380.2170(5-1) 520.2939 (0-2) 549.2725(0-3) 548.2885 (0-2) 213.1388 (2-0) 159.0922(3-0) 130.0655(3-0) 72.0808(3-0)
		-0.6	$[M-H]^-$	564.2824		HCD(50) CID(15)	564.2824(50-100) 180.0669(100-0) 202.0993(30-0)
	$C_{30}H_{43}N_5O_5$	1.4	$[M+Na]^+$	576.3165	21.0	HCD(20) CID(15)	576.3165(75-35) 312.1699(100-100) 411.2381(72-90) 284.1750(47-25) 185.1067(42-12) 265.1539(35-12) 158.0960(31-0) 254.1496(10-0) 364.2224(7-0) 196.0751(7-0) 558.3054(0-6) 530.3108(0-5) 72.0804(7-0)
		3.3	$[M-2H+Na]^-$	574.3030		HCD(50) CID(15)	574.3033(40-100) 164.0721(100-0)
	$C_{30}H_{43}N_5O_6$	0.2	$[M+Na]^+$	592.3107	15.5	HCD(20) CID(15)	592.3107(15-32) 261.1585(100-30) 304.1646(90-100) 289.1538(80-60) 287.1381(43-70) 475.2326(13-85) 159.0912(9-0) 493.2435(6-5) 574.3008(0-20) 411.2384(5-3) 162.0907(5-0) 312.1703(5-2) 546.3048(0-4) 241.1328(0-1)
	$C_{31}H_{41}N_5O_5$	-0.8	$[M-H]^-$	562.3030	19.2	CID(15)	562.3036(100)
	$C_{31}H_{41}N_5O_5$	-0.8	$[M-H]^-$	562.3030	21.4	CID(15)	562.3035(100)
	$C_{31}H_{42}O_6$	-1.1	$[M-H]^-$	531.2722	37.3	CID(15)	531.2715(100)
	$C_{31}H_{45}N_5$	-2.5	$[M+Na]^+$	510.3554	36.9	HCD(20) CID(10)	510.3561(10-100) 377.3194(100-15)
	$C_{31}H_{45}N_5$	-2.5	$[M+Na]^+$	510.3554	38.2	HCD(20) CID(10)	510.3561(20-100) 377.3193(100-5) 393.3388(0-20)
	$C_{32}H_{40}O_6$	-4.3	$[M+H]^+$	521.2875	39.5	HCD(20) CID(15)	521.2875(100-35) 131.0851(33-0) 475.2835(32-100) 205.1218(30-11) 299.1634(19-20) 471.2483(0-35) 485.2636(0-20) 461.2634(0-20) 443.2575(11-34) 489.2627(11-60) 503.2785(10-75)
	$C_{32}H_{42}O_7$	-3.6	$[M-H]^-$	537.2838	39.5	HCD(50) CID(15)	537.2845(10-100) 71.0507(100-0) 95.0507(60-0) 147.0821(40-0) 417.2808(52-0) 159.1184(27-0) 97.0663(35-0) 321.2232(15-0) 399.2707(15-0) 431.2970(20-0)177.1290(12-0)
	$C_{32}H_{42}O_8$	0.5	$[M-H]^-$	553.2810	38.3	HCD(50) CID(15)	553.2810(10-100) 121.0664(100-0) 97.0300(95-0) 175.1133(80-0) 201.0926(30-0) 219.1033(17-0) 393.2444(23-0)

$C_{32}H_{44}O_9$	-2.3	$[M-H]^-$	571.2899	36.8	HCD(50) CID(15)	571.2899(4-100) 87.0456(100) 175.1133(40-0) 363.2335(20-0) 439.2496(17-0) 113.0614(23-0) 121.0663(18-0)
$C_{34}H_{64}O_8$	-3.5	$[M-H]^-$	599.4507	39.2	HCD(50) CID(15)	599.4507(2-100) 317.2339(100-0)
$C_{38}H_{39}N_5O_7$	0.1	$[M-H]^-$	676.2778	36.9	HCD(50) CID(15)	676.2778(12-100) 330.0888(100-0)
$C_{38}H_{45}N_5O_6$	-4.1	$[M+H]^+$	668.3415	23.8	HCD(20) CID(15)	668.3415(17-100) 404.1962(100-25) 376.2011(62-18) 229.1330(50-12) 172.1113(4-0) 551.2631(0-2) 650.3317(0-5)
$C_{38}H_{45}N_5O_6$	-4.1	$[M+H]^+$	668.3415	24.2	HCD(20) CID(15)	668.3415(23-100) 650.3305(0-4) 503.2640(21-17) 404.1963(100-38) 376.2010(75-23) 277.1328(48-9) 220.1108(0-5)

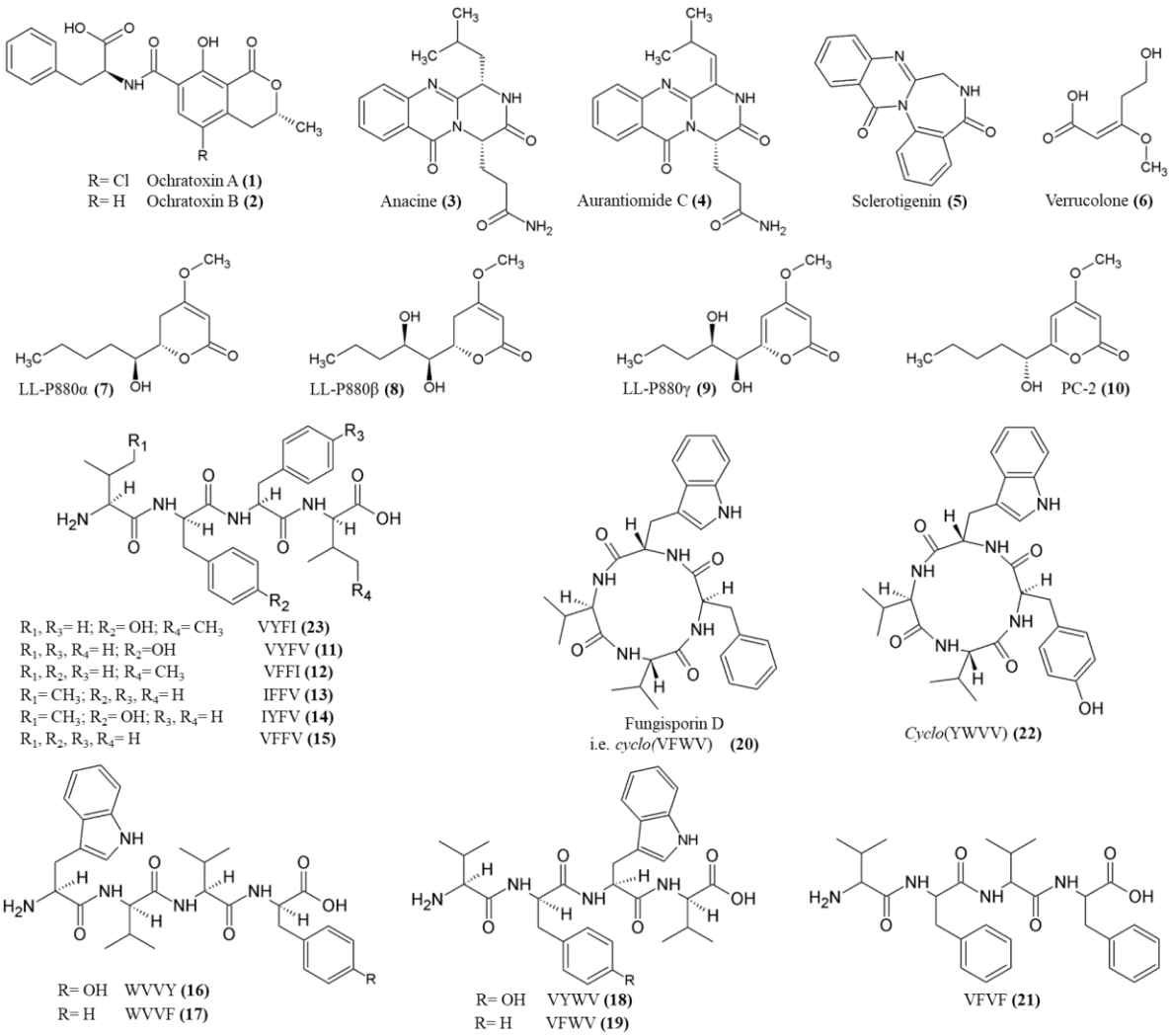

Figure S1. Structures of the secondary metabolites identified from *P. nordicum*.

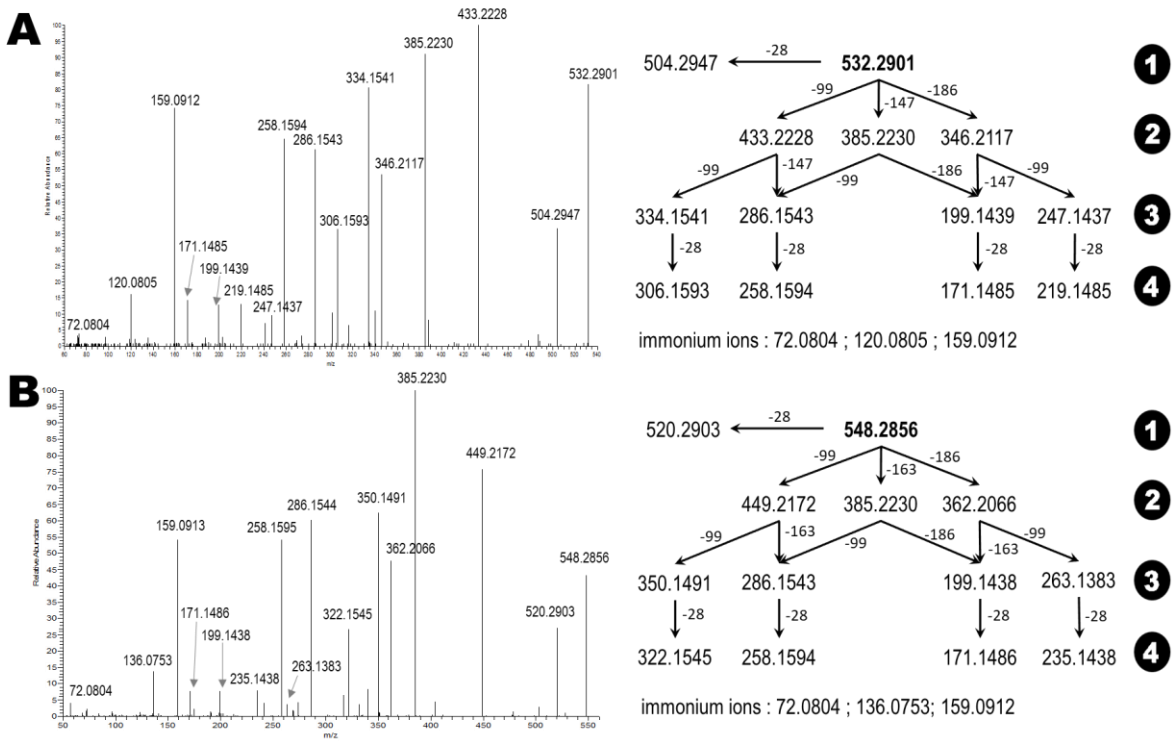

Figure S2.

HCD product ion spectra of (A) $[C_{30}H_{37}N_5O_4+H]^+$ detected at *m/z* 532.2901 and R_T 36.4 min, identified as fungisporin D (compound **20** in Figure S1) and (B) $[C_{30}H_{37}N_5O_5+H]^+$ detected at *m/z* 548.2845 and R_T 29.4 min, identified as *cyclo*(YWVV) (compound **22** in Figure S1). On the right side are presented the annotations of the product ions, resulting from ❶ a direct loss of carbon monoxide (illustrated as loss of 28 Da), ❷ the direct loss of one of the amino acid residue (b-like ions), ❸ the loss of two residues (b-like ions) or ❹ the loss of two residues and carbon monoxide (a-like ions). The immonium ions corresponded to losses of 99, 147, 163 and 186 Da representative of valine, phenylalanine, tyrosine and tryptophan residues, respectively.

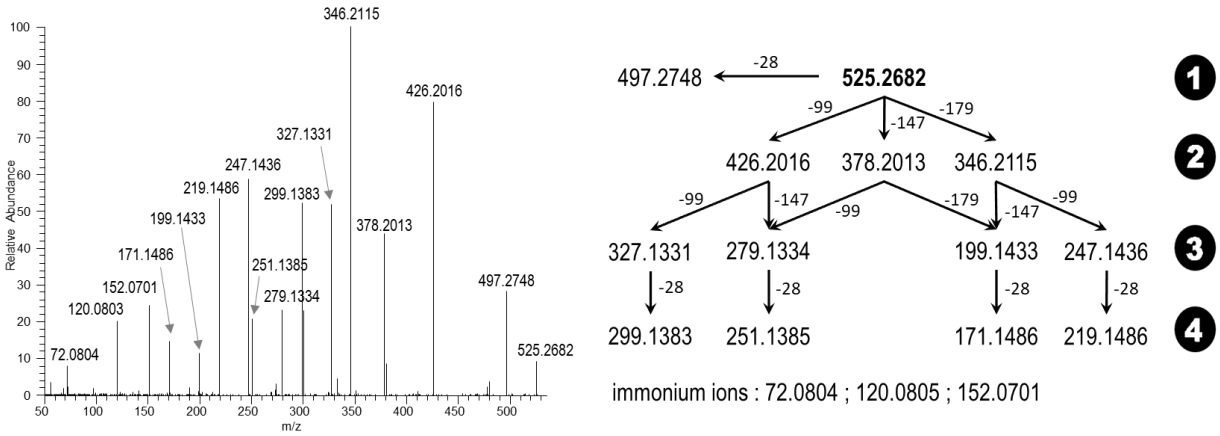

Figure S3. HCD product ion spectrum (NCE 20%) of $[C_{28}H_{36}N_4O_6+H]^+$ detected at *m/z* 525.2682 and R_T 25.8 min following the fragmentation pattern of the fungisporin D (Supplementary data Figure S2.A). On the right side are presented the annotations of the product ions, coming from ❶ a direct loss of carbon monoxide, ❷ the direct loss of one of the amino acid residue (b-like ions), ❸ the loss of two residues (b-like ions) or ❹ the loss of two residues and carbon monoxide (a-like ions). The immonium ions corresponded to losses of 99, 147 and 179 Da representative of valine, phenylalanine and putative hydroxylated tyrosine residues, respectively.

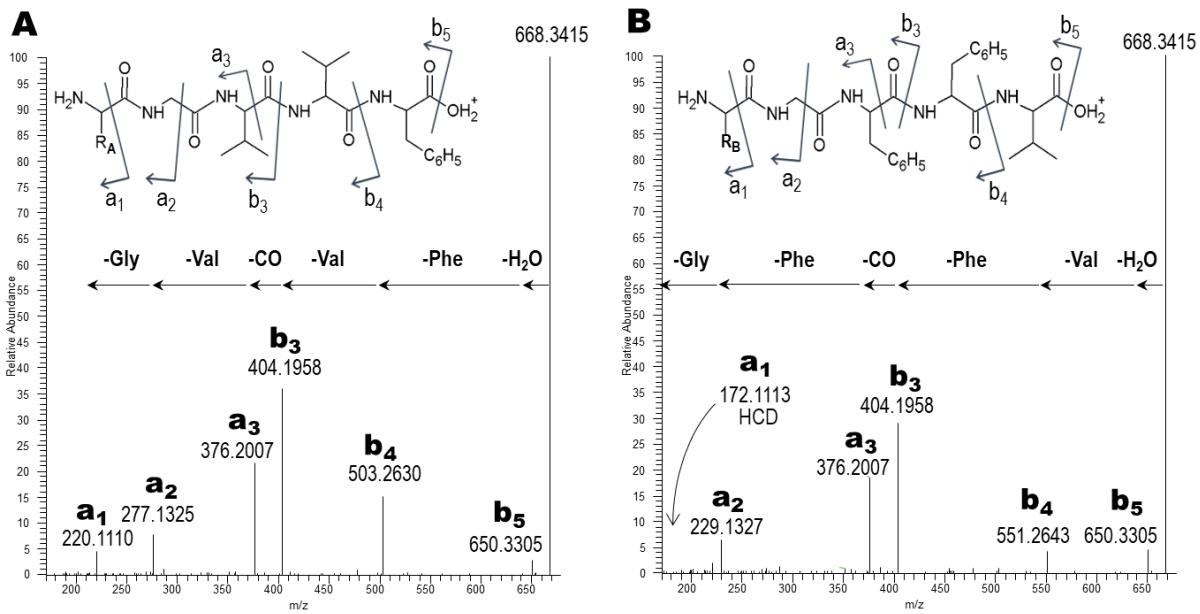

Figure S4.

Proposed interpretation of the high-resolution CID product ion spectra of the protonated peptides detected at *m/z* 668.3415 and (A) RT 4.2 min (compound **32** in Table 1) or (B) R_T 23.8 min (compound **33** in Table 1)

Table S2: Comparison of the sequences of the active sites of the adenylation domains detected by NRPSpredictor2 for HcpA in *P. rubens* (GenBank AID57164.1) and KOS44855.1 in *P. nordicum*. Different amino acid residues are highlighted in bold.

	Active sites of adenylation domains / stachelhaus code	Location in the sequence	Hypothetic substrate
First adenylation domain of the NRPS			
AID57164.1	GVHAFDASLAETCFVLAGEVNG YGPTEASVASVL / DAACVAGVAK	[401-534]	Hydrophobic-aromatic
KOS44855.1	GVHAFDASLAETCFVLAGEVNGY GPTEASVASVL / DAACVAGVAK	[216-349]	Hydrophobic-aromatic
Second adenylation domain of the NRPS			
AID57164.1	RAFASDAFIVEASIALGGEQVLYGTC ESSAASFH / DAVSAGLAAK	[1943-2077]	Apolar, aliphatic side chains
KOS44855.1	RAFASDAFIVEASIALGGEQVLYGTC ESSAASFH / DAVSAGLAAK	[1758-1892]	Apolar, aliphatic side chains
Third adenylation domain of the NRPS			
AID57164.1	TAMSFDVSI**L**EMSAITGGEINAYG PSEA**T**V**V**ATV / DV**L**SIGAV**V**K	[2737-2870]	Apolar, aliphatic side chains
KOS44855.1	TAMSFDVSI**M**EMSAITGGEINAYGPS EA**A**V**I**ATV / DV**M**SIGAV**I**K	[2553-2686]	Apolar, aliphatic side chains
Fourth adenylation domain of the NRPS			
AID57164.1	SAFAFDAAVMEAFLVCGGEMNG YGPTETCVFAV**I** / DAMFVGGVFK	[3843-3977]	Hydrophobic-aromatic
KOS44855.1	SAFAFDAAVMEAFLVCGGEMNGY GPTETCVFAV**V** / DAMFVGGVFK	[3660-3794]	Hydrophobic-aromatic

Table S3. Targeted analysis in the positive ESI mode of fungisporin-related metabolites and pentapeptides produced from *P. nordicum* grown on wheat, meet and cheese. Numbers in brackets refer to the number attached to each metabolite throughout the article. n.d. = not detected.

Peptides	MRM transitions	Detection in wheat grains	Detection in pork ham	Detection in turkey ham	Detection in raclette cheese
Fungisporin D (20)	532.3 > 433.2 532.3 > 504.3	Yes	n.d.	n.d.	n.d.
Cyclo(YWVV) (22)	548.3 > 449.2 548.3 > 520.3	Yes	Yes	n.d.	n.d.
Hydrolyzed Fungisporin A (15)	511.3 > 265.2 511.3 > 394.2	Yes	Yes	Yes	Yes
Hydrolyzed Fungisporin B (11)	527.3 > 265.2 527.3 > 410.2	Yes	Yes	Yes	Yes
Hydrolyzed Fungisporin D VFWV (19)	550.3 > 304.2 550.3 > 433.2	Yes	Yes	Yes	Yes
Hydrolyzed Fungisporin D WVVF (17)	550.3 > 286.2 550.3 > 385.2	Yes	Yes	Yes	Yes
Hydrolyzed *Cyclo*(VFVF) (21)	511.3 > 265.2 511.3 > 346.2	Yes	Yes	Yes	Yes
Hydrolyzed *Cyclo*(FFVI) (13)	525.3 > 265.2 525.3 > 408.2	Yes	Yes	Yes	Yes
Hydrolyzed *Cyclo*(FFIV) (12)	525.3 > 279.2 525.3 > 394.2	Yes	Yes	Yes	Yes
Hydrolyzed *Cyclo*(YFIV) (23)	541.3 > 279.2 541.3 > 410.2	Yes	n.d.	n.d.	n.d.
Hydrolyzed *Cyclo*(YFVI) (14)	541.3 > 265.2 541.3 > 424.2	Yes	Yes	Yes	Yes
Hydrolyzed *Cyclo*(YWVV) (18)	566.3 > 304.2 566.3 > 287.1	Yes	Yes	Yes	Yes
Hydrolyzed *Cyclo*(YWVV) (16)	566.3 > 286.2 566.3 > 385.2	Yes	n.d.	Yes	Yes
Cyclo(R_1VVF) (24)	525.3 > 426.2 525.3 > 497.3	Yes	Yes	Yes	Yes
Hydrolyzed *Cyclo*(VIVF) (25)	477.3 > 265.2 477.3 > 312.2	Yes	Yes	Yes	Yes
Hydrolyzed *Cyclo*(VR_2WV) (26)	592.3 > 304.2 592.3 > 287.1	Yes	n.d.	n.d.	n.d.
Hydrolyzed *Cyclo*(VR_2FV) (27)	537.3 > 273.2 537.3 > 372.2	Yes	n.d.	n.d.	n.d.
(28)	537.3 > 273.2	Yes	n.d.	n.d.	n.d.
(29)	537.3 > 273.2	Yes	n.d.	n.d.	n.d.
Hydrolyzed *Cyclo*(R_1FFV) (30)	539.3 > 265.2 539.3 > 422.2	Yes	n.d.	n.d.	n.d.
Hydrolyzed *Cyclo*(R_1R_2VF) (31)	576.3 > 312.2 576.3 > 411.2	Yes	Yes	Yes	Yes
R_1GVVF (32)	668.3 > 404.2 668.3 > 277.1	Yes	n.d.	n.d.	n.d.
R_1GFFV (33)	668.3 > 404.2 668.3 > 229.1	Yes	n.d.	n.d.	n.d.