

## Supporting information for

### **The *Penicillium brasilianum* histone deacetylase *clr3* regulates secondary metabolite production and tolerance to oxidative stress.**

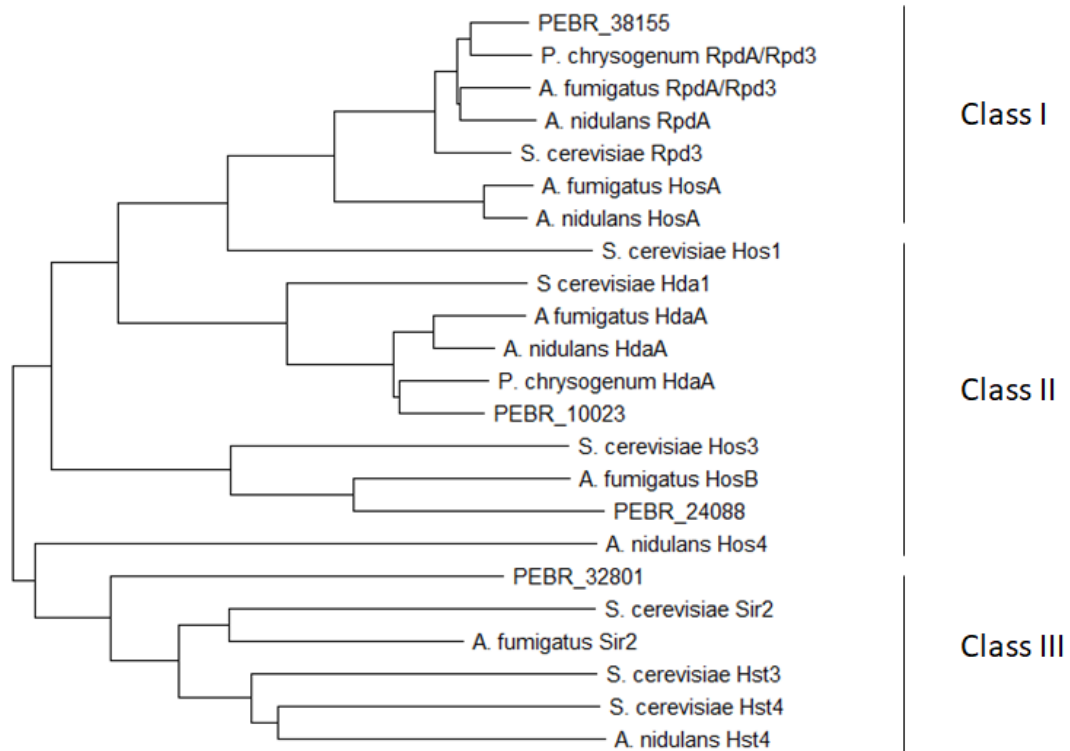
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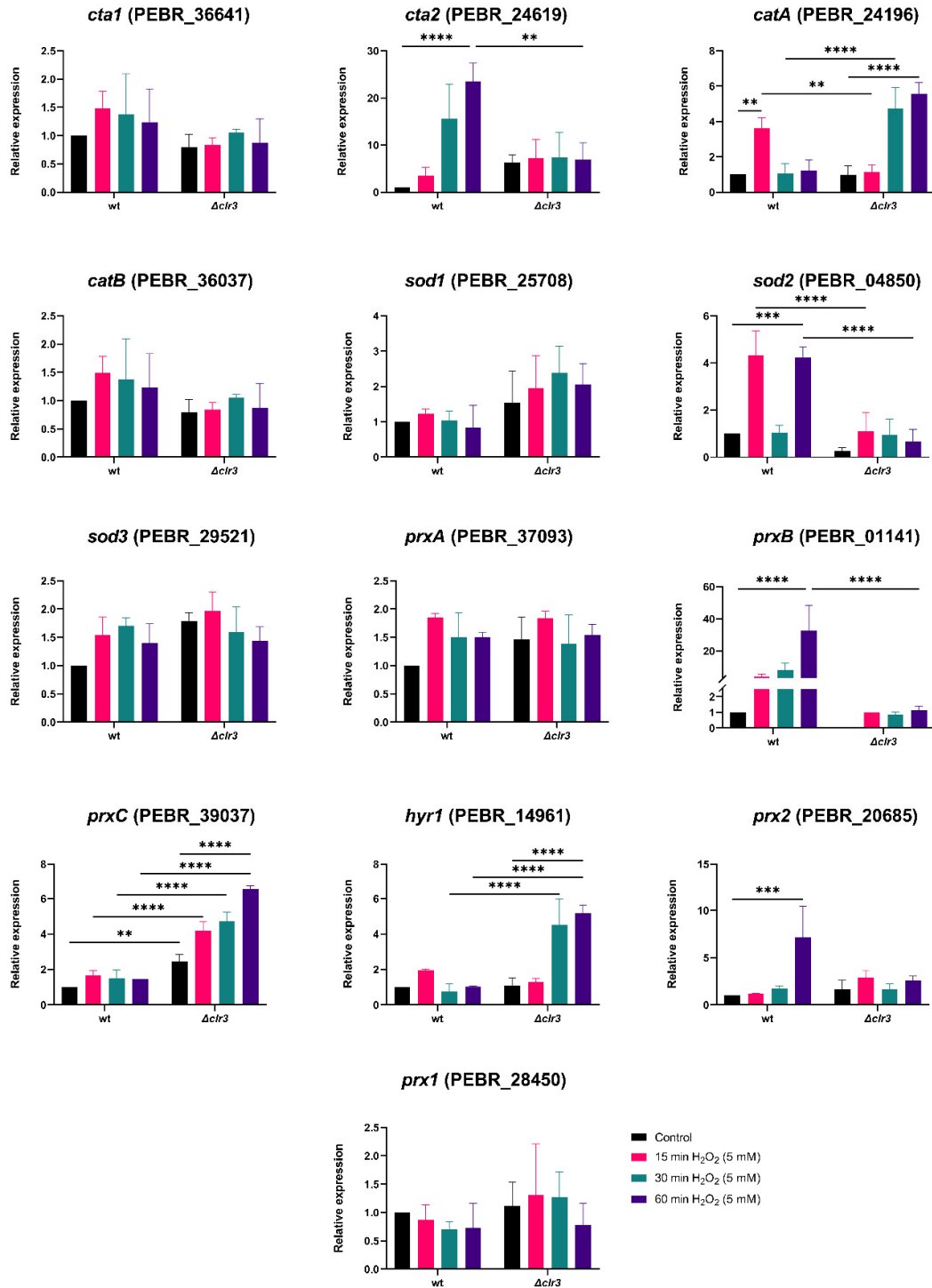
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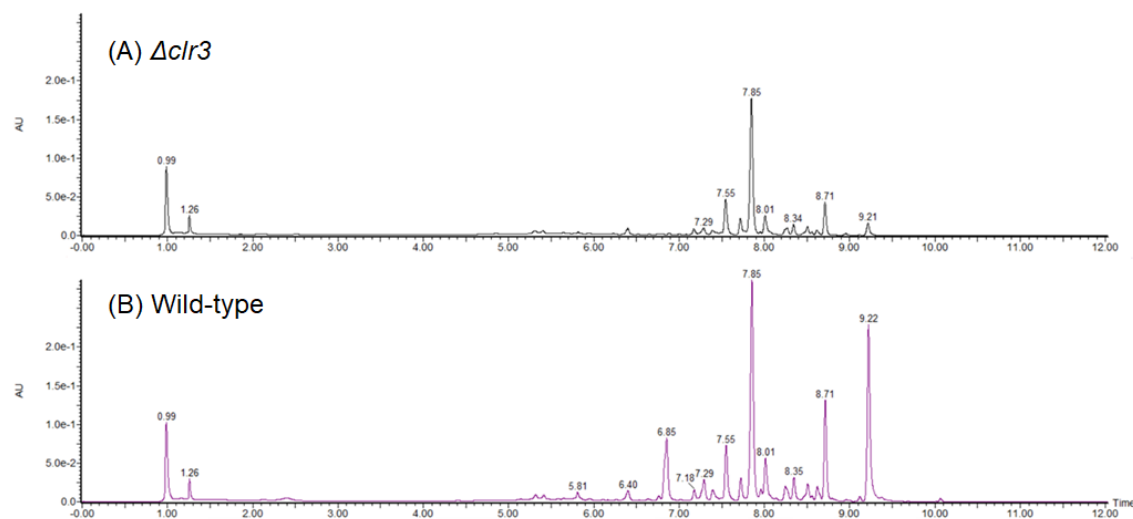


**Figure S1. Neighbor-joining phylogenetic tree of HDACs.** Histone deacetylase classifications are shown.

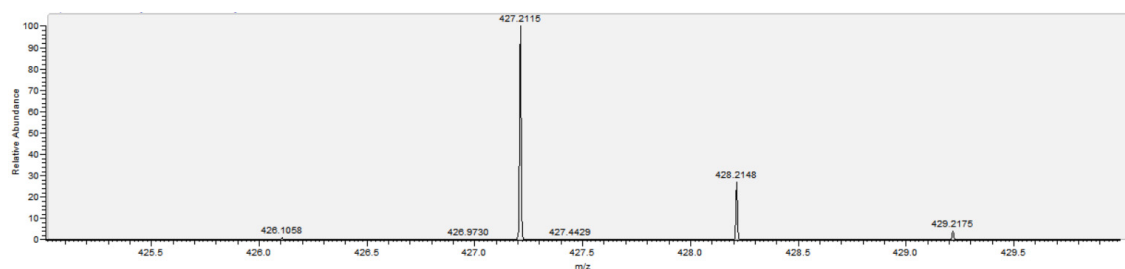


**Figure S2. Relative expression of genes encoding regulators of oxidative stress are different in the  $\Delta clr3$  mutant when compared to wild-type.** The fold increase in each strain represents the normalized mRNA abundance relative to the wild-type strain. The data represent the average value of at least three independent experiments with two technical

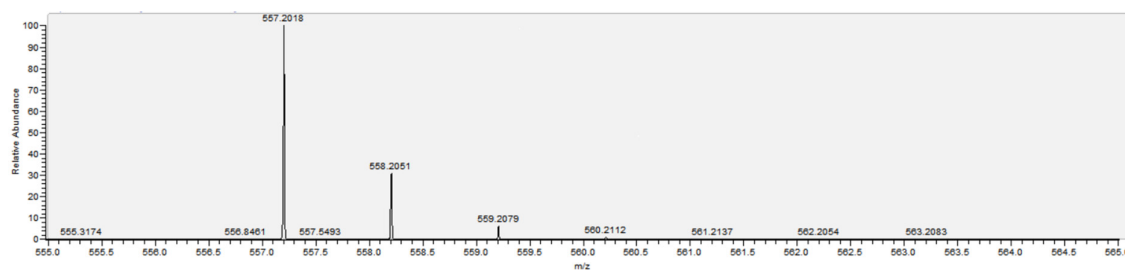
repetitions each. The error bars represent standard deviation, \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.005$ , \*\*\*\* $p \leq 0.001$  (two-way ANOVA – Tukey's test, significance level 0.05).



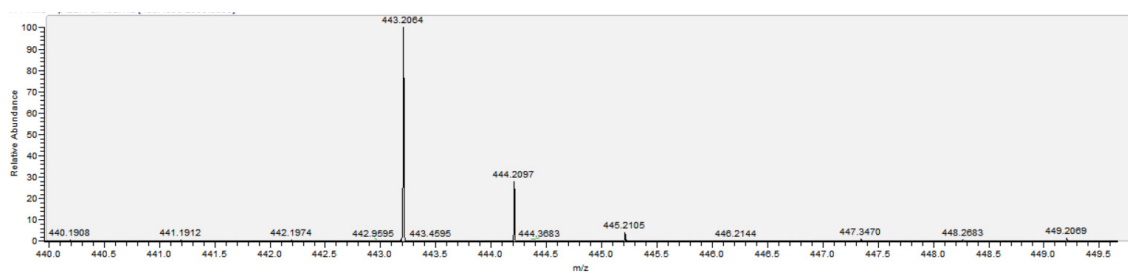
**Figure S3.** UPLC-DAD chromatograms obtained for the crude extracts from (A)  $\Delta clr3$  and (B) wild-type strains of *P. brasilianum*. Chromatograms were plotted to the same scale.



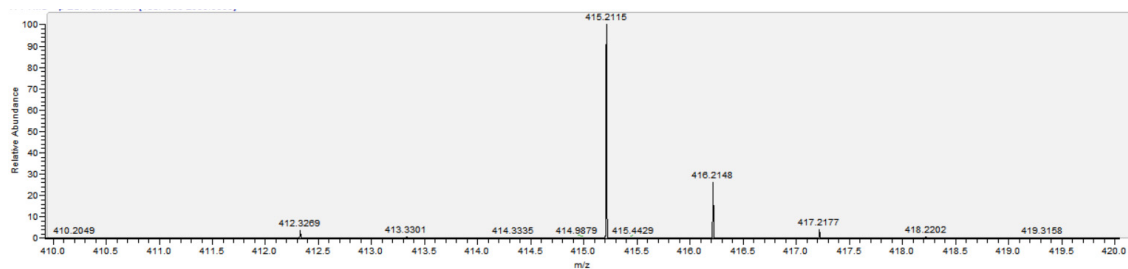
**Figure S4.** HRESI-MS data for isoaustinone.



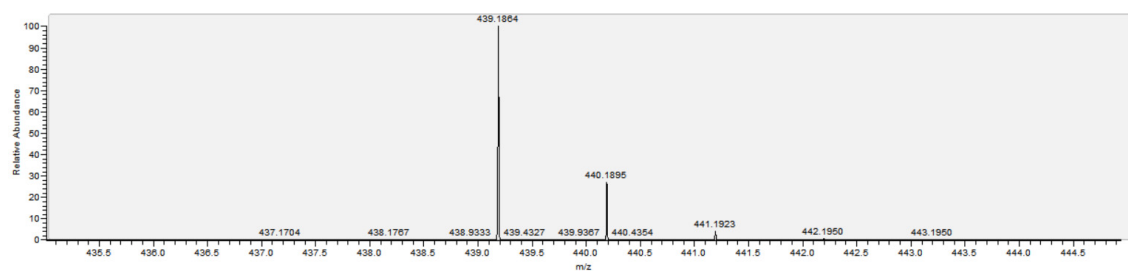
**Figure S5.** HRESI-MS data for acetoxyldehydroaustin.



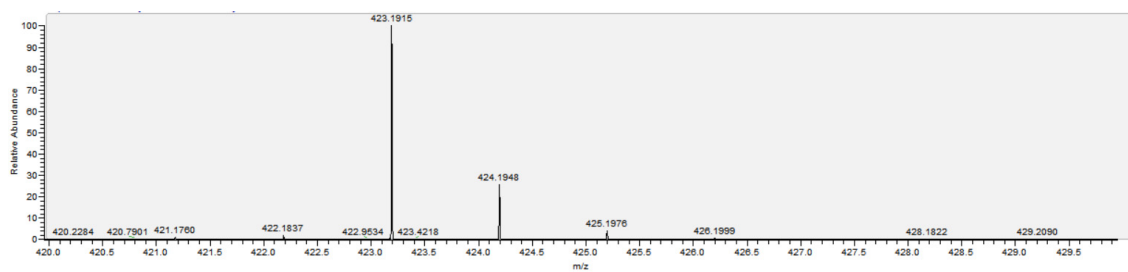
**Figure S6. HRESI-MS data for Austinol.**



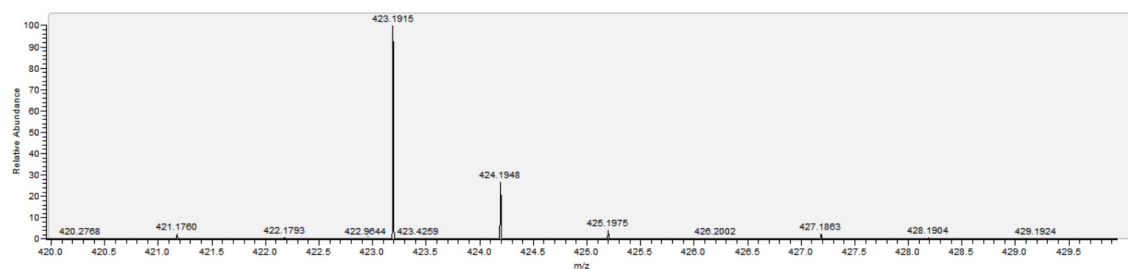
**Figure S7. HRESI-MS data for Austinoneol.**



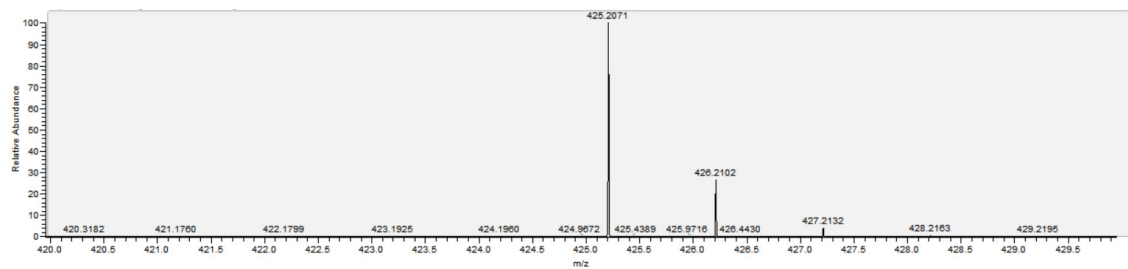
**Figure S8. HRESI-MS data for brasiliamide A.**



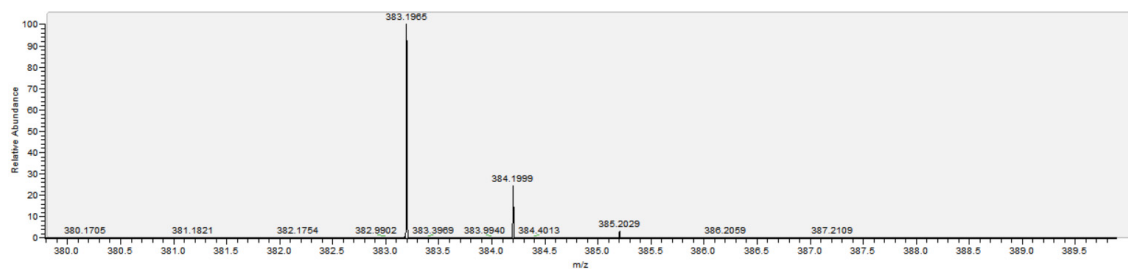
**Figure S9. HRESI-MS data for brasiliamide B.**



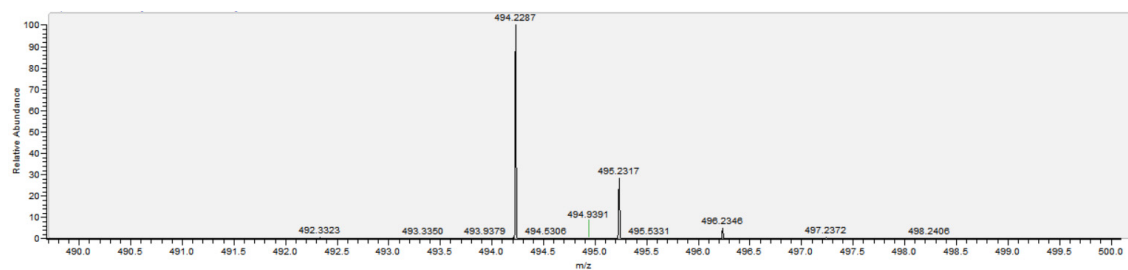
**Figure S10. HRESI-MS data for brasiliamide C.**



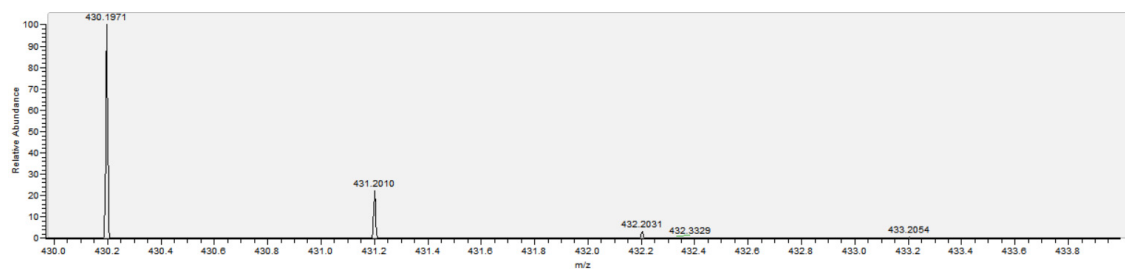
**Figure S11. HRESI-MS data for brasiliamide D.**



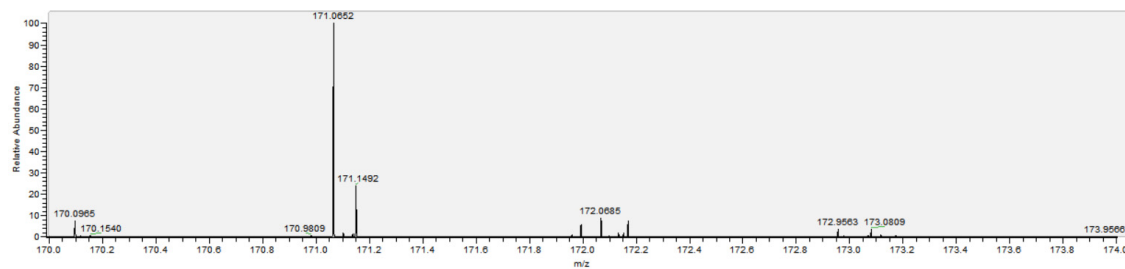
**Figure S12. HRESI-MS data for brasiliamide E.**



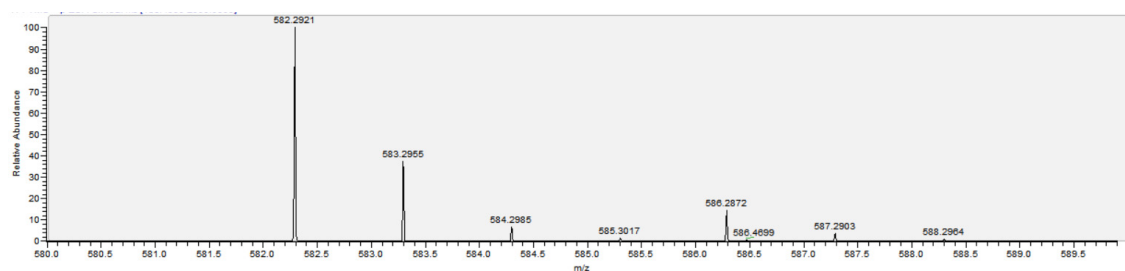
**Figure S13. HRESI-MS data for verruculogen.**



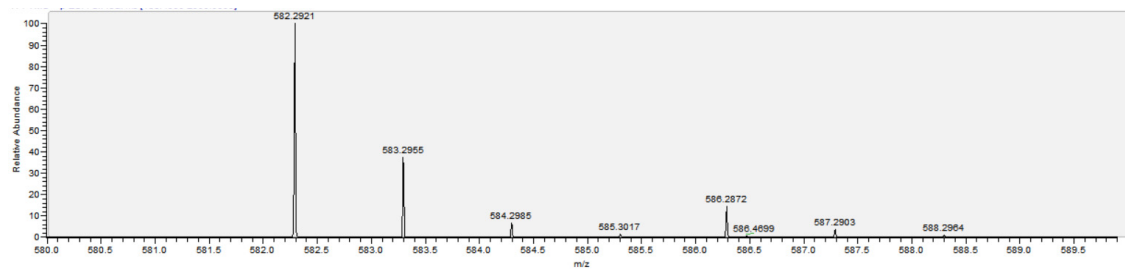
**Figure S14. HRESI-MS data for verruculogen TR-2.**



**Figure S15. HRESI-MS data for penicillic acid.**

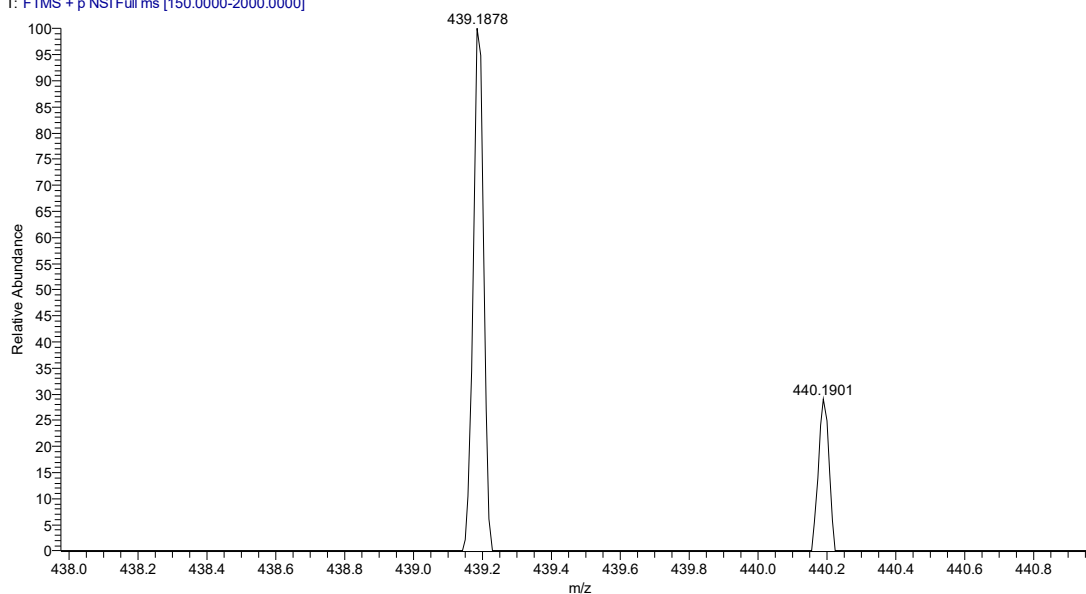


**Figure S16. HRESI-MS data for JBIR 114.**



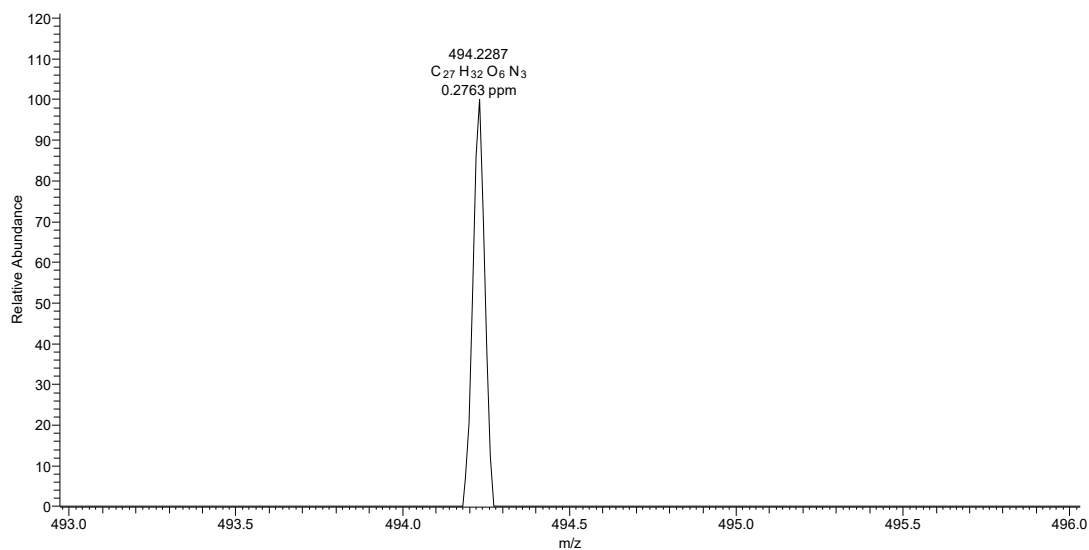
**Figure S17. HRESI-MS data for JBIR 115.**

Dani\_WTxDELTA43 #40 RT: 0.15 AV: 1 NL: 9.68E3  
T: FTMS + p NSI Full ms [150.0000-2000.0000]



**Figure S18. Mass spectrum of ion  $[M+H]^+$   $m/z$  439.1878 obtained for brasiliamide A through DESI-IMS.**

Dani\_WTxDELTA61 #66 RT: 0.25 AV: 1 NL: 3.17E3  
T: FTMS + p NSI Full ms [150.0000-2000.0000]



**Figure S19. Mass spectrum of ion  $[M+H]^+$   $m/z$  494.2287 obtained for verruculogen through DESI-IMS.**



**Table S1.** Primers used in this study for construction of  $\Delta clr3$  strain.

Primer name	Sequence (5' → 3')
clr3 5F	<u>GTAACGCCAGGGTTTTCCCAGTCACGACGAGCTTCGGTCGAAAGGTA</u>
clr3 5R	tcagctaggtcaacgaataaggatgattctTTGCCAGGTGGAGAGAAG
hph F	AGAATCATCCTTATTCGTTGACCT
hph R	AGGTCGAGTGGAGATGTGGA
clr3 3F	aagcgccactccacatctccactcgacctGTTGAAGCTAACGCCATC
clr3 3R	<u>GCGGATAACAATTTTACACAGGAAACAGCCGAAGCAATAATCCATCG</u>
clr3 500 ups	TCCGTCGTTGACCTGACTTG
clr3 F	CGTCAAGCGCGATAGTTTGG
clr3 R	GATCATCCACCAACCCAGCA

Small letters indicate homology to hygromycin gene fragment in the cassette.  
Underlined letters indicate homology to the pRS426 flanking sequence.

**Table S2.** *Penicillium brasilianum* strains used in this study.

Name	Genotype	Reference
Wild-type	Wild-type (LaBioMMi 136)	(dos Santos Geris and Rodrigues-Fo 2002)
$\Delta clr3$	$\Delta clr3::hph$ ; Hyg <sup>R</sup>	This study

**Table S3.** Individual genes selected for RT-qPCR analyses based on the orthology with oxidative stress regulation genes in *A. fumigatus*.

Gene	<i>A. Fumigatus</i> ortholog	Identity (%)	Similarity (%)	E-Value	DeepLoc prediction (likelihood)
<i>prx1</i> (PEBR_28450)	AFUB_065670	83	90	$7.10^{-133}$	Cytoplasm (0.6905)
<i>prx2</i> (PEBR_20685)	AFUB_080670	78	86	$7.10^{-152}$	Mitochondrion (0.993)
<i>prxA</i> (PEBR_37093)	AFUB_096050	85	90	$8.10^{-102}$	Cytoplasm (0.8825)
<i>prxB</i> (PEBR_01141)	AFUB_078510	80	90	$2.10^{-104}$	Mitochondrion (0.998)
<i>prxC</i> (PEBR_39037)	AFUB_049980	79	88	$4.10^{-96}$	Cytoplasm (0.9428)
<i>hyr1</i> (PEBR_14961)	AFUB_036900	76	87	$7.10^{-106}$	Cytoplasm (0.6753)

<i>sod1</i> (PEBR_25708)	AFUB_056780	87	97	$3.10^{-98}$	Mitochondrion (0.4173)
<i>sod2</i> (PEBR_04850)	AFUB_014080	68	77	$5.10^{-97}$	Cytoplasm (0.6944)
<i>sod3</i> (PEBR_29521)	AFUB_073150	82	90	0	Mitochondrion (0.9843)
<i>cta1</i> (PEBR_36641)	AFUB_017280	43	58	$6.10^{-128}$	Peroxisome (0.7115)
<i>cta2</i> (PEBR_24619)	AFUB_033720	44	57	$4.10^{-133}$	Peroxisome (0.7445)
<i>catA</i> (PEBR_24196)	AFUB_094400	64	77	0	Cytoplasm (0.5587)
<i>catB</i> (PEBR_36037)	AFUB_046060	51	65	$2.10^{-153}$	Extracelullar (0.8947)

**Table S4.** Primers used in this study for RT-qPCR analyses.

Gene	Primer name	Sequence (5' → 3')
<i>prx1</i> (PEBR_28450)	PEBR_28450 F	ACCCCGAGCGCAAGGT
	PEBR_28450 R	CGTTGGTGGTGTCTGCTAGT
<i>prx2</i> (PEBR_20685)	PEBR_20685 F	TGCCTTCCTGTACGACATGATT
	PEBR_20685 R	TGCCCTTTTGGTCGATGTTAT
<i>prxA</i> (PEBR_37093)	PEBR_37093 F	CCGCTGACCGCTACAATCTC
	PEBR_37093 R	GTGGTTGGCAGAGCAAACG
<i>prxB</i> (PEBR_01141)	PEBR_01141 F	AGGGCGACGCCATTCC
	PEBR_01141 R	TTGTTGCCGGGAGAGTTCTC
<i>prxC</i> (PEBR_39037)	PEBR_39037 F	GGTCAGCTATGCGCAAATAGAGA
	PEBR_39037 R	ACGGGATGCTCCTACCTTGA
<i>hyr1</i> (PEBR_14961)	PEBR_14961 F	GCTTCCCCTGCAACCAGTT
	PEBR_14961 R	GGACTGGATCTCATCGTTGGA
<i>sod1</i> (PEBR_25708)	PEBR_25708 F	TGGCTCCAAGCAGGACAAG
	PEBR_25708 R	CGCCAAGGACGCTCTCA
<i>sod2</i> (PEBR_04850)	PEBR_04850 F	GCATCAATTGCCCCTCAA
	PEBR_04850 R	GGTTCAAGGGCCTGAAGGTAA
<i>sod3</i> (PEBR_29521)	PEBR_29521 F	CACCGCATCCCCCAATT
	PEBR_29521 R	TCCGGAACACCATTCTCCTT
<i>cta1</i> (PEBR_36641)	PEBR_36641 F	CGCCACATGAACGGCTACT
	PEBR_36641 R	GGTACCGTCCGGCTTGATC
<i>cta2</i> (PEBR_24619)	PEBR_24619 F	TATCGCCTCGGCGTGAAC
	PEBR_24619 R	GGACCTTGGCCGCGTTA
<i>catA</i> (PEBR_24196)	PEBR_24196 F	ACCAAGCGCAAACCTTCTACA
	PEBR_24196 R	TTGGCAACGTGCAGCTTTT
<i>catB</i> (PEBR_36037)	PEBR_36037 F	CGCTGGATGATGCCAGAT
	PEBR_36037 R	GAACGTGAGTAGTCCTTCCTTC
<i>clr3</i> (PEBR_10023)	PEBR_10023 F	CCACCTGATCGCCTCAAGAT
	PEBR_10023 R	TGACGAGATTCCAGGGAAT