

Suppressive effects of volatile compounds from *Bacillus* spp. on *Magnaporthe oryzae* *Triticum* (MoT) pathotype, causal agent of wheat blast

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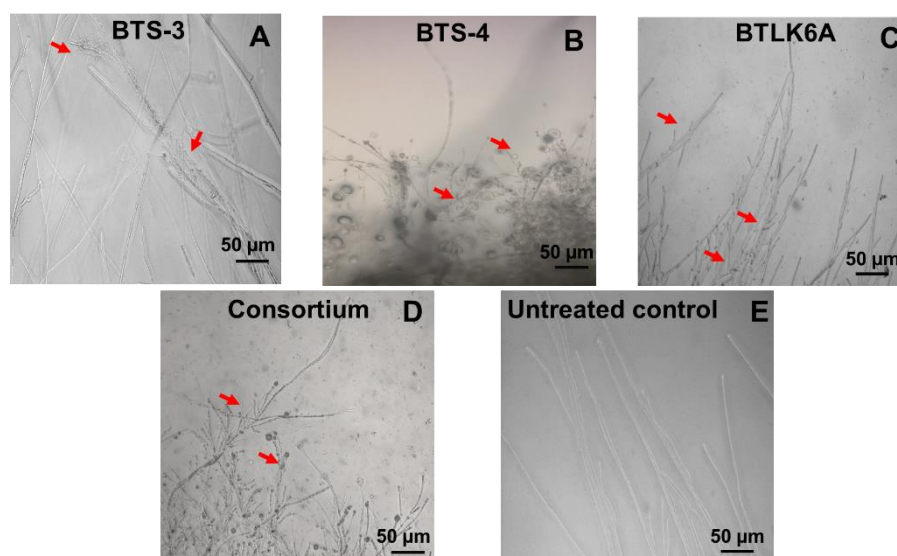
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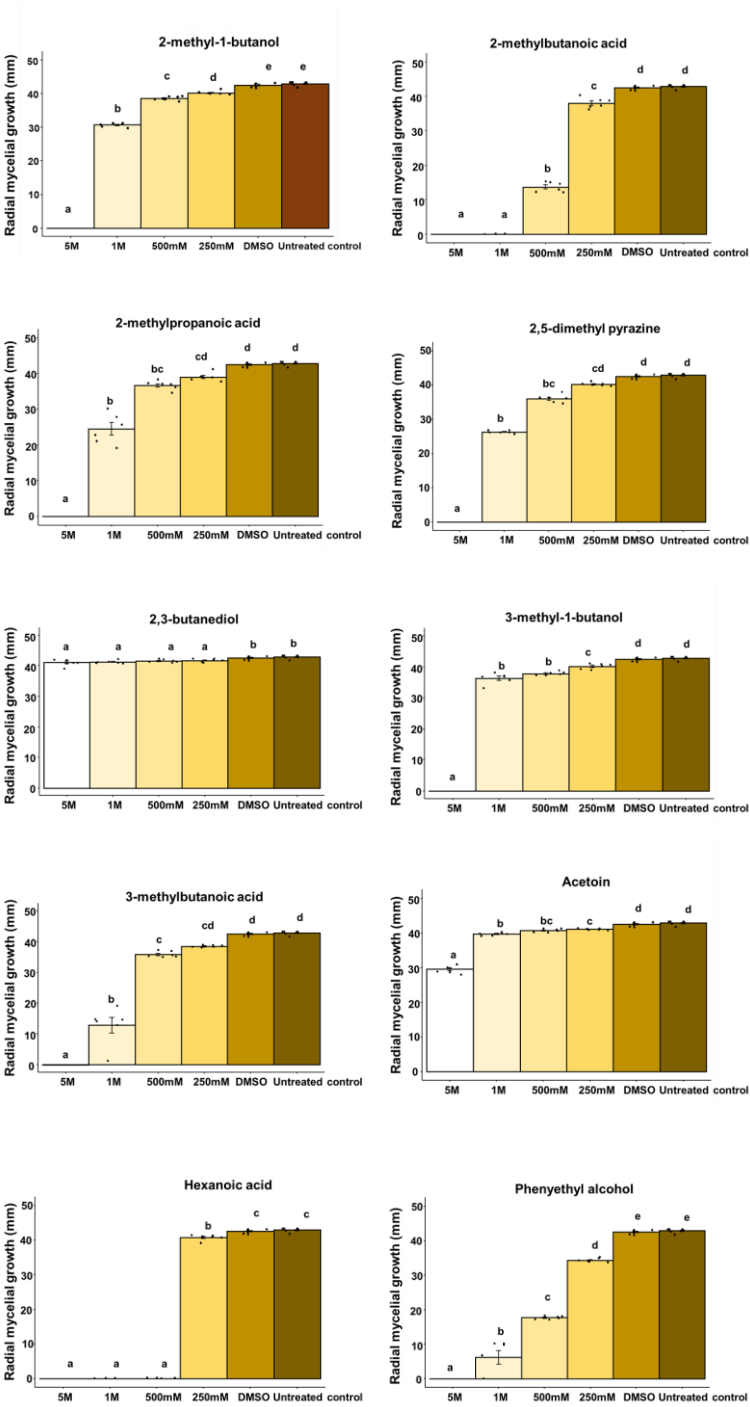
Supplementary Materials

Supplementary figure S1.



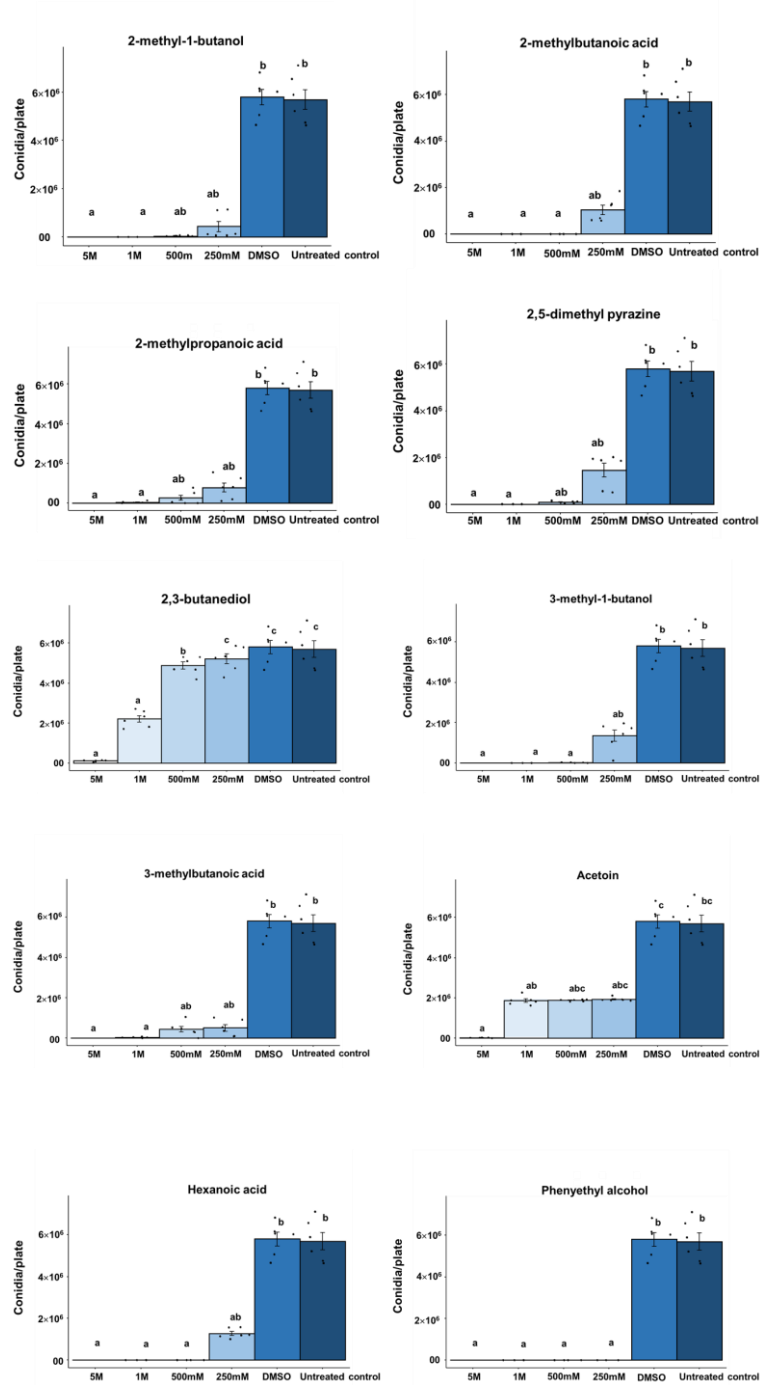
Supplementary figure S1. Alternation of MoT mycelial morphology of representative samples caused by antifungal VOCs from *Bacillus* spp. Pictures were taken at 5 d after MoT inoculation at 25°C (10X magnification). Where, BTS-3: *B. subtilis* BTS-3, BTS-4: *B. amyloliquefaciens* BTS-4, BTLK6A: *B. amyloliquefaciens* BTLK6A, consortium: a mixture of BTS-3, BTS-4 and BTLK6A; and untreated control: water.

Supplementary figure S2.



Supplementary figure S2. Assessment of pure VOCs on MoT mycelial growth in upside-down Petri dish assay *in vitro*. Data were recorded 5 d after MoT inoculation at 25°C (Dunn test; n = 6; $p \leq 0.05$). Each data point represents each replication within the same sampling data set.

Supplementary figure S3.



Supplementary figure S3. Assessment of pure VOCs on sporulation of MoT in upside-down Petri dish assay *in vitro*. Data were recorded 5 d after MoT inoculation at 25°C (Dunn test; n = 6; $p \leq 0.05$). Each data point represents each replication within the same sampling data set.

Supplementary table S4: Details of purchased pure volatile compounds

Chemical name	CAS number	Molar mass (g/mol)	Company
2-Methylpropionic acid (99%)	79-31-2	88.11	SIGMA, sigma-aldrich Chemie GmbH, Riedstr. 2 D-89555, Steinheim 49 7329 970
3-Methyl-1-butanol (98%)	123-51-3	88.15	SIGMA, sigma-aldrich Chemie GmbH, Riedstr. 2 D-89555, Steinheim 49 7329 970
3-Methylbutanoic acid (99%)	503-74-2	102.13	SIGMA, sigma-aldrich Chemie GmbH, Riedstr. 2 D-89555, Steinheim 49 7329 970
2,3-Butanediol (98%)	513-85-9	90.12	SIGMA, sigma-aldrich Chemie GmbH, Riedstr. 2 D-89555, Steinheim 49 7329 970
2-Methylbutyric acid (98%)	116-53-0	102.132	SIGMA, sigma-aldrich Chemie GmbH, Riedstr. 2 D-89555, Steinheim 49 7329 970
Phenylethyl alcohol (≥99%)	60-12-8	122.167	SIGMA, sigma-aldrich Chemie GmbH, Riedstr. 2 D-89555, Steinheim 49 7329 970
Hexanoic acid (≥98%)	142-62-1	116.16	SIGMA, sigma-aldrich Chemie GmbH, Riedstr. 2 D-89555, Steinheim 49 7329 970
2-Methyl-1-butanol (≥99%)	137-32-6	88.15	SIGMA, sigma-aldrich Chemie GmbH, Riedstr. 2 D-89555, Steinheim 49 7329 970
2,5-Dimethylpyrazine (≥98%)	123-32-0	108.14	SIGMA, sigma-aldrich Chemie GmbH, Riedstr. 2 D-89555, Steinheim 49 7329 970
Acetoin (95%)	513-86-0	88.105	SIGMA, sigma-aldrich Chemie GmbH, Riedstr. 2 D-89555, Steinheim 49 7329 970

Supplementary table S5: Identification and quantification of bacterial VOCs

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Compound	RI (L)	RI (E)	BTS-3	BTS-4	BTLK6A	Consortium
			ng/h/ml	ng/h/ml	ng/h/ml	ng/h/ml
*Acetoin	706.0	706.5	-	0.911	-	-
*1-Butanol, 3-methyl-	734.0	731.8	-	0.181	-	0.015
*Pyrazine, 2,5-dimethyl-	911.0	912.4	1.081	0.026	0.600	0.825
*1-Butanol, 2-methyl-	736.0	736.8	-	0.023	-	-
*Propanoic acid, 2-methyl-	793.0	797.4	0.152	0.047	0.038	0.027
*2,3-Butanediol	782.0	783.3	-	0.429	-	0.004
*Butanoic acid, 3-methyl-	866.0	868.5	0.324	0.303	0.465	0.058
*Butanoic acid, 2-methyl-	886.2	883.3	0.580	0.667	0.537	0.081
*Hexanoic acid	993.0	992.3	0.009	0.009	-	-
*Phenyl ethyl alcohol	1120.0	1123.1	-	0.023	-	0.004
2-Heptanone	895.0	893.0	0.011	0.020	-	0.011
Heptanal	904.0	903.8	-	0.009	-	-
1-Hexene, 4,5-dimethyl-	970.0	900.0	-	-	-	0.049
2-Heptanone, 6-methyl-	932.0	900.5	0.023	-	0.033	0.023
Benzaldehyde	921.0	900.6	0.005	-	-	0.003
2-Heptanone, 5-methyl-	974.0	999.0	0.025	0.009	0.018	0.005
2 Methyl pyrazine	826.0	820.0	-	0.062	-	-
Pyrazine, 2-ethyl-3-methyl-	999.0	991.1	0.003	-	-	-
Octanal	1003.0	1004.6	-	0.013	-	-
Pyrazine, trimethyl-	989.0	1000.0	0.092	0.014	0.040	0.044
2-Acetylthiazole	1014.0	1022.0	0.010	0.005	0.009	0.007
1-Hexanol, 2-ethyl-	1029.0	1030.3	0.042	0.020	0.015	0.004
Benzyl alcohol	1035.7	1037.2	-	0.010	-	0.011
Undecane	1000.0	1000.0	0.007	0.005	-	-
Acetophenone	1065.0	1071.5	0.012	0.011	-	0.006
Phenol, 2-methoxy-	1096.0	1093.0	-	0.125	-	-
2-Nonanol	1102.0	1100.0	-	0.017	-	-
Nonanal	1104.0	1105.0	0.015	0.025	-	0.006
2-Decanone	1191.0	1100.0	-	0.004	-	-
1-Decene	991.0	993.0	-	0.005	-	-
Dodecane	1200.0	1200.0	-	0.003	0.003	-
Decanal	1205.0	1206.9	-	0.003	-	-
Benzothiazole	1221.0	1201.0	0.008	0.004	0.007	0.004
Nonanoic acid	1272.0	1263.4	-	0.018	-	-
2-Undecanone	1294.0	1295.3	-	0.014	-	-
1-Pentanol	744.1	741.9	-	-	-	0.008

Hexanal	811.0	810.7	0.012	0.091	-	-
3-Pentanol	814.0	800.0	-	0.055	-	-
2-Hydroxy-3-pentanone	821.0	800.2	-	0.049	-	-
Pyrazine, 2-methyl-	853	859.3	-	0.014	-	0.011
Diethyl acetic acid	885	846.7	-	1.083	-	-

Where, BTS-3= *B. subtilis* BTS-3; BTS-4= *B. amyloliquefaciens* BTS-4; BTLK6A= *B. amyloliquefaciens* BTLK6A; consortium=a mixture of BTS-3, BTS-4 and BTLK6A; *= Gold identified by using pure compound; RT = Retention time; RI (L) = Retention Index (Literature); RI (E) = Retention Index (Experimental)

Supplementary table S6: Effect of pure VOCs to suppress MoT *in vitro*

Compound	Concentration	Mycelial radial growth (mm)	Total conidia/plate
2-Methyl-1-butanol	5M	0.0 ± 0.0	0.0
	1M	30.7 ± 0.2	0.0
	500mM	38.5 ± 0.2	3.2×10 ⁴
	250mM	40.1 ± 0.1	4.2×10 ⁵
2-Methylbutanoic acid	5M	0.0 ± 0.0	0.0
	1M	0.0 ± 0.0	0.0
	500mM	13.7 ± 1.5	0.0
	250mM	38.0 ± 0.6	1.1×10 ⁶
2-Methylpropanoic acid	5M	0.0 ± 0.0	0.0
	1M	24.0 ± 1.7	4.2×10 ⁴
	500mM	36.7 ± 0.4	2.6×10 ⁵
	250mM	39.0 ± 0.4	7.8×10 ⁵
2,5-Dimethyl pyrazine	5M	0.0 ± 0.0	0.0
	1M	26.33 ± 0.2	0.0
	500mM	36.0 ± 0.4	8.6×10 ⁴
	250mM	40.2 ± 0.1	1.5×10 ⁶
2,3-Butanediol	5M	41.0 ± 0.4	1.1×10 ⁵
	1M	41.2 ± 0.2	2.2×10 ⁶
	500mM	41.5 ± 0.2	4.8×10 ⁶
	250mM	41.7 ± 0.2	5.2×10 ⁶
3-Methyl-1-butanol	5M	0.0 ± 0.0	0.0
	1M	36.33 ± 0.7	0.0
	500mM	37.8 ± 0.3	1.5×10 ⁴
	250mM	40.2 ± 0.4	1.4×10 ⁶
3-Methylbutanoic acid	5M	0.0 ± 0.0	0.0
	1M	12.8 ± 2.5	3.3×10 ⁴
	500mM	35.5 ± 0.4	4.4×10 ⁵
	250mM	38.5 ± 0.2	5.0×10 ⁵

Acetoin	5M	29.5 ± 0.4	2.7×10^4
	1M	39.7 ± 0.2	1.7×10^6
	500mM	40.7 ± 0.2	1.8×10^6
	250mM	41.0 ± 0.0	1.9×10^6
Hexanoic acid	5M	0.0 ± 0.0	0.0
	1M	0.0 ± 0.0	0.0
	500mM	0.0 ± 0.0	0.0
	250mM	40.7 ± 0.3	1.3×10^6
Phenylethyl alcohol	5M	0.0 ± 0.0	0.0
	1M	6.2 ± 2.0	0.0
	500mM	17.6 ± 0.2	0.0
	250mM	34.3 ± 0.2	0.0
DMSO	-	42.5 ± 0.2	5.8×10^6
Untreated control	-	42.8 ± 0.1	5.7×10^6