ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Biocontrol Activity of *Bacillus megaterium* BM344-1 against Toxigenic Fungi

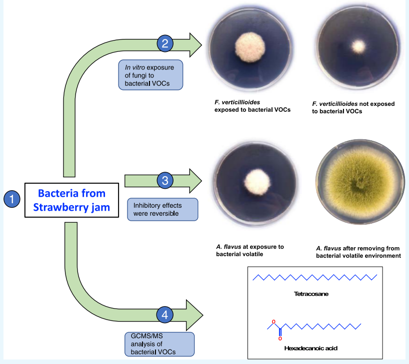
Aya Ehab Saleh, Zahoor Ul-Hassan, Randa Zeidan, Noora Al-Shamary, Thoraya Al-Yafei, Hajer Alnaimi, Nayla Salah Higazy, Quirico Migheli, and Samir Jaoua*

Cite This: *ACS Omega* 2021, 6, 10984−10990

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Mycotoxins are secondary metabolites of some fungal species and represent important contaminants of food and feed. This study aimed to explore the biological control activity of *Bacillus megaterium* BM344-1 volatile organic compounds (VOCs) on the growth and mycotoxin production of single representatives of the toxigenic species *Aspergillus flavus*, *Aspergillus carbonarius*, *Penicillium verrucosum*, and *Fusarium verticillioides*. *In vitro* co-incubation experiments indicated the *P. verrucosum* isolate as the most sensitive one, with a growth inhibition ratio of 66.7%, followed by *A. flavus* (29.4%) and *F. verticillioides* (18.2%). Exposure of *A. flavus*, *P. verrucosum*, and *F. verticillioides* to BM344-1 VOCs resulted in complete inhibition of aflatoxins (AFB_1, AFG_1, and AFG_2), ochratoxin A, and fumonisin B_1 (FB_1) synthesis on artificial media, respectively. *In vivo* experiments on maize kernels showed 51% inhibition of fungal growth on ears simultaneously infected with *A. flavus* spores and exposed to BM344-1 volatiles. Likewise, AF synthesis by *A. flavus* was significantly ($p < 0.05$) inhibited (25.34 ± 6.72 μg/kg) by bacterial volatiles as compared to that in control maize ears (91.81 ± 29.10 μg/kg). Gas chromatography−tandem mass spectrometry-based analysis of headspace volatiles revealed hexadecanoic acid methyl ester (palmitic acid) and tetracosane as bioactive compounds in the BM344-1 volatilome. Bacterial volatiles have promising potential to control the growth and mycotoxin synthesis of toxigenic fungi and may present valuable aid in the efforts to warrant food and feed safety.



1. INTRODUCTION

Mycotoxins are important contaminants of agriculture and food industries and are mainly produced by some species of *Aspergillus*, *Penicillium*, and *Fusarium*. After the first discovery of aflatoxin (AF) in 1960s, there has been a tremendous effort to dissect mycotoxin nature, toxicity, and mycotoxigenic species.[1] At present, the list of known mycotoxins covers over 400 compounds,[2] including toxins produced by *Aspergillus* and *Penicillium* (such as AFs, ochratoxins, patulin, *etc.*) and *Fusarium* (*e.g.*, zearalenone, fumonisins, deoxynivalenol, and T-2/HT-2). AFB_1 produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* is widely known for its hepatotoxicity[3] and has been classified as a group 1A human carcinogen.[4] Ochratoxin A (OTA), a nephrotoxic metabolite, is found in many food commodities and is synthesized by some *Aspergillus* (*Aspergillus carbonarius*, *Aspergillus ochraceous*, *Aspergillus westerdijkiae*, *Aspergillus niger*, *etc.*) and *Penicillium* (such as *Penicillium verrucosum* and *Penicillium nordicum*) species.[5] Fumonisins (FB_1 and FB_2) are among the most important mycotoxins produced by *Fusarium* species (*Fusarium verticillioides* and *Fusarium proliferatum*) and induce neurotoxic effects on the exposed animal and human.[6]

Pre- and post-harvest contamination of food crops with toxigenic fungi and the accumulation of their toxins remain ever challenging for food and feed regulatory authorities.[7] Agricultural husbandry practices such as crop rotation, proper sowing and harvesting timing, insect and pest control, grading and segregation of products, proper irrigation and the use of effective fungicides result in significant control of fungal infection and mycotoxin accumulation.[8,9] However, persistence of fungicide residues in food[10] and emerging fungicide-resistant fungal populations[11] are major concerns associated with chemical fungicides. Likewise, some physical control methods in spite of having significant potential to degrade mycotoxins may affect the quality of cereal-derived food and feed. UV irradiation of toxin-contaminated food not only has limited applicability but also compromises the nutritional and organoleptic characteristic of food.[12]

Over the recent past, several efforts have been devoted to define alternate and safer strategies to minimize the impact of mycotoxins and to control fungal infection in crops. Microbial

Received: February 14, 2021
Accepted: April 2, 2021
Published: April 13, 2021

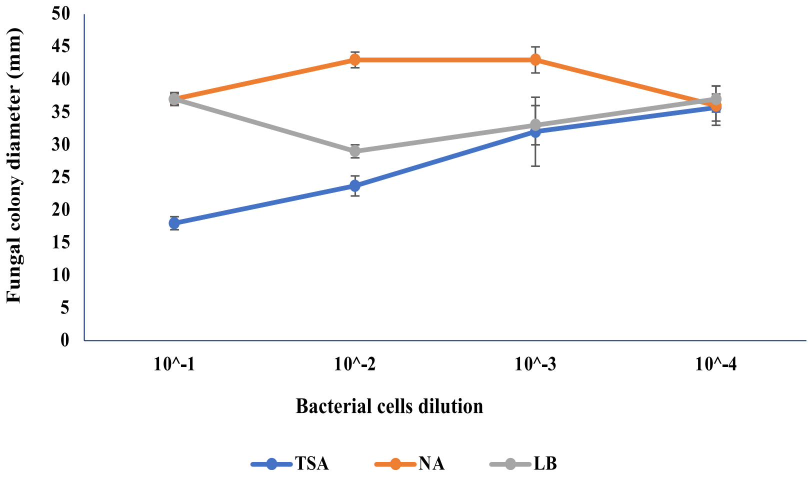


Figure 1. Effect of the type of growth media and bacterial cell dilution on the antifungal activity of *B. megaterium* BM344-1. Bacterial cells at dilutions 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were spread inoculated on three types of media (TSA, LB agar, and NA) and sealed with fungal inoculated plates.

control by living and inactivated yeasts and bacterial cells, their diffusible and volatile compounds, and enzymes are being explored for their antagonistic potential against fungi.[13,14]

In our previous studies, we have reported yeast[15−18] and bacterial[19−21] cultures possessing strong antifungal potential against toxigenic fungi. This study was designed to investigate *in vitro* as well as *in vivo* effects of *Bacillus megaterium* (BM344-1) against the growth and toxin production potential of toxigenic isolates of *A. flavus*, *P. verrucosum*, and *F. verticillioides*. Additionally, the chemical nature of the BM344-1 volatilome was investigated to identify the bioactive molecule (s) in the bacterial volatilome.

2. RESULTS AND DISCUSSION

2.1. Optimum Conditions for the Efficient Production of Bacterial Antifungal Volatiles. Lipophilicity, low polarity, high vapor pressure, and low molecular weight are the main characteristics of microbial volatile organic compounds (VOCs) that are gaining momentum for their potential application against fungal contamination of food and feed commodities.[22,23] The precise mode of action of VOCs is not yet well understood and probably varies with the chemical nature of molecules and their microbial sources.[24] However, interference with the fungal metabolic pathways by alteration in the expression of key genes is generally an accepted mechanism of their antifungal activity.[15,16] Three media [tryptic soy agar (TSA), Luria-Bertani (LB), and nutrient agar (NA)] and four bacterial cell dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) were preliminarily tested to explore the appropriate requirements for an efficient antagonistic activity of BM344-1 against *Aspergillus carbonarius* AC82. The volatiles produced by BM 344-1 on TSA at 10^{-1} dilution showed the highest inhibitory effect on a colony size of *A. carbonarius* as measured at day 7 of co-incubation (Figure 1). The composition of growth media, particularly protein- and sugar-contents, plays a key role in the bacterial volatilome. On protein-rich media, *Lysobacter* sp. produced bioactive compounds such as pyrrole, decanal, and pyrazines as compared to inactive compounds on sugar-rich media.[25] In the present study, the antagonistic activity of BM344-1 was linked to protein richness with the highest inhibitory efficacy measured on TSA (15 g of pancreatic casein and 5 g of soy peptone in 1 L), followed by that on LB (10 g of tryptone, 5 g of yeast extract), and the least on NA (5 g of peptone and 2 g of yeast extract). In the study of Bruce *et al.*,[26] VOCs produced by bacterial cultures on TSA showed a complete inhibition of fungal growth, whereas inhibition was minimal when bacteria were grown on other media. In fact, amino acids acting as components of antagonistic volatiles are found in particular high-protein media compared to others.

2.2. Antagonistic Spectrum of *B. megaterium* BM344-1 Volatiles against *A. flavus*, *F. verticillioides,* and *P. verrucosum*. Exposure of different mycotoxigenic fungi to BM344-1 volatiles resulted in a significant decrease in the colony diameter as compared to that of unexposed control fungi. *P. verrucosum* showed the highest sensitivity to bacterial volatiles, followed by *A. flavus* and *F. verticillioides*. The growth inhibition ratios (%) calculated with comparison to control fungi were 66.7, 29.4, and 18.2% for *P. verrucosum*, *A. flavus*, and *F. verticillioides*, respectively (Figure 2). The higher sensitivity of *P. verrucosum* compared to that of *A. flavus* to bacterial volatiles was previously observed by Ul Hassan *et al.*[19]

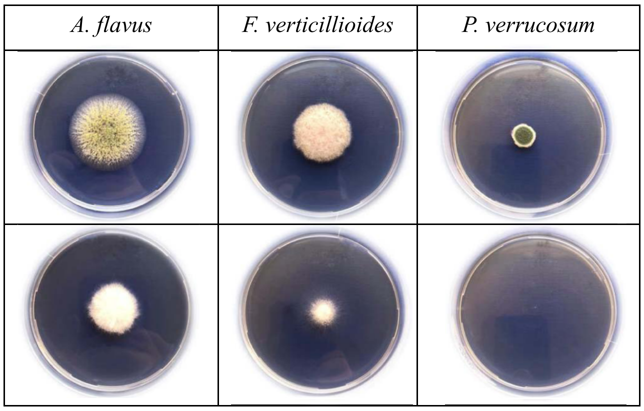


Figure 2. Spectrum of antifungal activities of *Bacillus megaterium* BM344-1 against toxigenic *Aspergillus*, *Fusarium*, and *Penicillium* fungi. The fungi in the second row are the control (unexposed to bacterial volatiles), while those in the third row showing significant effects on colony size and sporulation are exposed to *B. megaterium* BM344-1 volatiles for 3 days.

Exposure of *P. verrucosum* and *A. flavus* to *Bacillus licheniformis* volatiles (the major antagonistic compound was 3-methyl-1-butanol) resulted in 53 and 49% reduction in the colony diameter when compared to that of the unexposed control, respectively. In line with this study, Zeidan *et al.*[17] found that the highest sensitivity is of *Penicillium*, followed by that of *Aspergillus,* and the least by *Fusarium* to yeast VOCs. The observed differences in fungal colony diameters among the three fungi (each from different genus) in response to bacterial volatiles may be associated with their cell wall structure. The cell wall composition of fungi varies according to their microenvironmental stressors and plays a significant role in the fungal resistance.[27,28] Antagonistic *Bacillus* volatiles (such as those of *Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus cereus,* and *B. megaterium*) against phytopathogenic and toxigenic *Aspergillus* and *Penicillium* spp. have been reported by several authors.[29−31]

2.3. Reversibility in BM344-1-Induced Fungal Growth Inhibition.

After removal from the bacterial volatile environment, all three fungi showed normal growth and sporulation, suggesting that microbial volatiles effects were transient and the presence of antagonistic bacteria or their VOCs is needed for consistent inhibition. In a study by Wheatley *et al.*,[32] similar reversibility to physiological growth and sporulation was observed in fungi after removal from the bacterial environment. In a similar study, after removal from the VOC environment, Fiori *et al.*[33] observed the reversibility of sporulation in *A. carbonarius* which was completely inhibited upon exposure to yeast volatiles.

B. licheniformis BL350-2 producing 3-methyl-1-butanol as a bioactive compound caused significant growth inhibition in *Aspergillus westerdijkiae* BA1 (62%), *A. carbonarius* MG7 (60%), *P. verrucosum* MC12 (53%), *Aspergillus niger* MC05 (50%), *A. flavus* CM5 (49%), *A. parasiticus* SB01 (47%), and *Aspergillus ochraceus* MD1 (44%), which showed complete reversal upon removing the fungi from the bacterial VOC environment.[19]

2.4. Inhibitory Effect of *B. megaterium* BM344-1 on Mycotoxin Synthesis.

Exposure to BM344-1 volatiles not only inhibited the vegetative growth but also affected the mycotoxin biosynthesis potential of toxigenic fungi (Table 1). At day 7 of co-incubation, *A. flavus* showed a significant reduction in AFB_2 synthesis, while the production of other classes of AFs (AFB_1, AFG_1, and AFG_2) was totally inhibited. Similarly, OTA synthesis by *P. verrucosum* and FB_1 by *F. verticillioides* were also completely inhibited by bacterial volatiles. *F. verticillioides* exposed to BM344-1 was able to synthesize FB_2, but the concentration of this mycotoxin in the medium was significantly lower than that of unexposed control fungi.

Table 1. Effect of *B. megaterium* BM344-1 Volatiles on the Mycotoxin Biosynthesis by Different Toxigenic Fungi[a]

fungi	mycotoxin (μg/kg)	control	VOCs-exposed
A. flavus	AFB_1	199.44 ± 16.40^a	n.d*
	AFB_2	84.82 ± 11.00^a	13.91 ± 2.45^b
	AFG_1	37.26 ± 4.50^a	n.d
	AFG_2	14.21 ± 2.12^a	n.d
P. verrucosum	OTA	84.80 ± 9.50^a	n.d
F. verticillioides	FB_1	1.04 ± 0.07^a	n.d
	FB_2	11.85 ± 2.36^a	1.62 ± 0.01^b

[a] Effect of *B. megaterium* BM344-1 volatiles on mycotoxin production of *A. flavus, P. verrucosum,* and *F. verticillioides.* Mycotoxin production of the control (fungi not exposed to BM344-1 volatiles) and VOCs-exposed fungi are shown as mean ± SD obtained from three replicates. Different superscript letters on values in rows indicate the significant difference at $p \leq 0.05$. *Not detected (below the limit of detection of the analytical system).

The inhibition/reduction in mycotoxin synthesis by toxigenic fungi in the bacterial[19,30] or yeast[15−18] VOCs-saturated environment could be associated with changes in the expression of biosynthetic-cluster genes,[15] protein profiles,[16,34] or altered enzymatic activities of the target fungi.[32] The volatiles synthesized by *B. megaterium* KU143 resulted in the inhibition of AF accumulation on stored rice grains colonized by *A. flavus.*[30]

2.5. Biological Control Activity of *B. megaterium* BM-344-1 against *A. flavus* Growth and AF Synthesis on Maize Kernels.

In vivo exposure of *A. flavus*-infected maize ears to BM3441-1 volatiles showed a significant inhibition of fungal growth as well as AF synthesis (Table 2). In the control maize ears (without BM344-1 VOCs), the spread of *A. flavus,* measured as number of kernels with visible fungal growth, was significantly higher (22.5 ± 0.7 kernels) compared to that in the ears infected with fungi and exposed to bacterial VOCs (11 ± 1 ears), showing 51% inhibition in the fungal growth as a consequence to bacterial VOC exposure. In line with the present study, Mannaa *et al.* (2017)[30] reported a significant decrease in the *A. flavus* population on un-hulled rice grains exposed to *B. megaterium* KU143 volatiles. The VOCs (3-methyl-1-butanol as compound) produced by *B. licheniformis* showed a similar inhibitory effect on the growth of *A. flavus* on infected maize ears.[19]

Table 2. *In Vivo* Antifungal Activity of *B. megaterium* BM344-1 Volatiles on Infected Maize Ears[a]

treatment	no. of infected kernels (growth inhibition ratio)	AF (μg/kg)
control (exposed to BM344-1)	0	n.d*
BM344-1 +*A. flavus*	11 ± 1.0^b (51%)	25.34 ± 6.72^b
TSA +*A. flavus*	22 ± 0.0^a(2%)	99.85 ± 36.21^a
A. flavus	22.5 ± 0.7^a(0%)	91.81 ± 29.10^a

[a] Surface-disinfected maize ears were inoculated with *A. flavus* spores and exposed to *B. megaterium* BM344-1 volatiles. The values in each column represent the mean ± SD of three replicates, and the different superscript letter indicates the significant difference at $p \leq 0.05$. *n.d = not detected.

In line with the fungal growth, the levels of AFs in the VOCs-exposed *A. flavus*-contaminated maize ears were significantly ($p \leq 0.05$) lower [25.34 ± 6.72 ± standard deviation (SD)] than that in the unexposed maize ears (91.81 ± 29.10). TSA alone showed no effect on fungal growth and its mycotoxin production ability (Table 2). These *in vivo* results are in line with the *in vitro* antagonistic activity of BM344-1 against mycotoxin synthesis potential of *A. carbonarius, P. verrucosum,* and *F. verticillioides* (Section 4.4). Inhibition of AF synthesis by *A. flavus* on exposure to volatiles emitted by *B. megaterium* KU143 and *B. licheniformis* 350-2 on un-hulled rice and maize ears has been reported by Mannaa and Kim[31] and Ul Hassan *et al.*,[19] respectively. However, in the present study, it is unclear if the observed reduced mycotoxin synthesis is associated with fungal growth slowdown or with specific

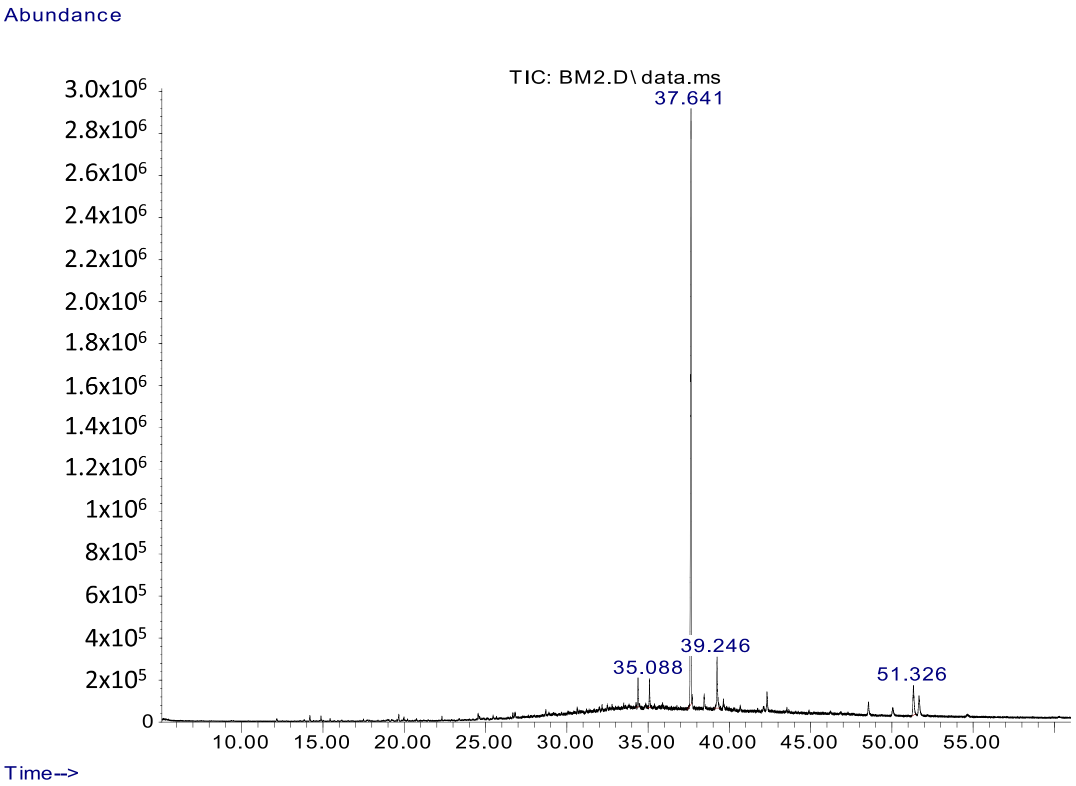


Figure 3. GC−MSMS chromatograph of detected compounds in *B. megaterium* BM344-1 headspace volatiles. On the *x*-axis, there is retention time in min, and on the *y*-axis, there is retention time in abundance of compounds. The compound detected at 35.08 min is tetracosane and that at 39.24 min is hexadecanoic acid methyl ester. The peaks in chromatographs from BM344-1-inoculated headspace volatiles were compared with those of the control (media without bacterial inoculation). The peaks detected at 37.64 and 52.32 min were found in both the control and bacterial inoculated headspace volatiles, probably indicating the compounds emitted by the media.

mechanisms such as effects on the expression of genes involved in mycotoxin biosynthesis[15] or alteration in the enzymatic activities.[32]

2.6. GC−MSMS Analysis of BM344-1 Volatiles.

Bacterial volatiles analysis performed by gas chromatography−mass spectrometry (GC−MS) revealed the presence of hexadecanoic acid methyl ester (palmitic acid) and tetracosane. Both these compounds are well-known microbial volatiles holding strong antagonistic activities against toxigenic as well as phytopathogenic fungi (Figure 3).[33−35]

The absence of these compounds in the control flasks [tryptic soy broth (TSB) without bacteria] suggests that both the fungal growth and mycotoxin synthesis inhibition were due to single or synergistic/additive interaction of the two compounds (Table 3). Hexadecanoic acid was the major compound in the microbial VOC mix of *Bacillus atrophaeus* HAB-5[36] inhibiting *Colletotrichum gloeosporioides*[35] and seaweeds suppressing *Aspergillus*, *Penicillium*, and *Fusarium*.[37] Likewise, tetracosane was the major constituent of antifungal volatiles produced by *Chaetomium globosum*.[38]

Table 3. GC−MS Analysis of *B. megaterium* BM344-1 Headspace Volatiles[a]

S. no.	name	retention time (min)	peak area (%)
1	hexadecanoic acid methyl ester	39.24	8.18
2	tetracosane	35.08	3.41

[a] Detected volatile compounds with a peak area of less than 1.5% are not listed in this table. The compounds detected in *B. megaterium* BM344-1 headspace volatiles as well as in the control flasks (containing TSA only) are also excluded.

3. CONCLUSIONS

The volatiles produced by *B. megaterium* BM344-1 have shown high potential against the growth and mycotoxin biosynthesis in three representative isolates of *A. flavus*, *P. verrucosum*, and *F. verticillioides*. The antifungal activity of BM344-1 was enhanced by increasing the protein content in the growth medium. During *in vitro* co-incubation experiments, *P. verrucosum* showed the highest sensitivity, with a growth inhibition ratio of 66.7%, followed by *A. flavus* (29.4%) and *F. verticillioides* (18.2%). Exposure of *A. flavus*, *P. verrucosum*, and *F. verticillioides* to BM344-1 VOCs resulted in complete inhibition of AFs (AFB_1, AFG_1, and AFG_2), OTA, and fumonisin B_1 (FB_1) synthesis on artificial media, respectively. Under *in vivo* testing on maize ears, BM344-1 showed significant inhibition of *A. flavus* growth and AF synthesis on infected kernels. The headspace analysis of bacterial volatiles indicated hexadecanoic acid methyl ester (palmitic acid) and tetracosane as bioactive compounds. These results suggest potential application of bacterial culture for the preservation of food commodities.

4. MATERIALS AND METHODS

4.1. Microbial Cultures and Growth Media.

B. megaterium BM344-1 was isolated from strawberry jam (imported from Turkey) marketed in Qatar and identified by its protein spectrum using matrix-assisted laser desorption ionization time-of-flight.[19] *A. flavus* CECT 2687 was obtained from the culture collection center, University de Valencia Spain; *A. carbonarius* AC82, *F. verticillioides* FV04, and *P. verrucosum* PV11 were isolated from animal feed.[22] TSA was prepared by adding 15 g of pancreatic casein, 5 g of soy peptone, 5 g of sodium chloride, and 15 g of agar in 1 L of distilled water. LB agar for *Bacillus* sp. was prepared by mixing 15 g of agar, 10 g of tryptone, 5 g of yeast extract and 5 g of NaCl in 1 L of distilled water. NA was prepared by adding peptone (5 g), meat extract (1 g), yeast extract (2 g), sodium chloride (5 g), and agar (15 g) in 1 L of distilled water.

4.2. Optimization and Investigation of *B. megaterium* BM344-1 for Its Antifungal Activities. In order to find appropriate conditions for the optimal production of antifungal volatiles, different dilutions of *B. megaterium* BM344-1 were preliminarily inoculated on three types of bacterial growth media. In each case, 100 μL of bacterial cell suspension [10^{-1} (~2.5×10^7 cfu/mL), 10^{-2} (~2.5×10^6 cfu/mL), 10^{-3} (~2.5×10^5 cfu/mL), 10^{-4} (~2.5×10^4 cfu/mL), and 10^{-5} (~2.5×10^3 cfu/mL)] was plated on TSA, LB, and NA. Inoculated plates were incubated at 30 °C for 24 h. In an Eppendorf tube, fungal spores of *A. carbonarius* were prepared by transferring inocula from the freshly sporulating fungal colony to 1 mL of saline solution, amended with 0.05% Tween 80. A 10 μL aliquot of the spore suspension (adjusted at $\times 10^4$) was inoculated at the center of PDA plates. The cover of the fungal inoculated plates was replaced with the base plate of bacterial inoculated plates. The two plates were sealed face-to-face with a double layer of Parafilm and then an additional layer of scotch tape. The sealed plates were incubated at 26 °C for 72 h before measuring the diameter of the fungal colonies and the extent of sporulation. Fungal growth inhibition was calculated as

$$\text{fungal growth inhibition (\%)} = \frac{(C - T)}{C}100$$

C = colony diameter (mm) of control fungi. T = colony diameter (mm) of fungi exposed to bacterial volatiles.

After optimization, the spectrum of antifungal activities of BM344-1 was tested on three fungi (*A. flavus, F. verticillioides,* and *P. verrucosum*) representing different genera. In each case, 100 μL of 10^{-1} bacterial dilution was applied on TSA.

4.3. Reversibility of Bacterial VOC Effects on the Mycotoxigenic Fungi. To explore the reversibility of the effects of bacterial volatiles on fungal growth, at day 7 of exposure, a plug of ~1 cm^2 was removed from the margin of the fungal colony with a sterile blade and transferred to a new PDA plate. The inoculated plates were incubated at 26 °C to check the fungal growth and sporulation. The fungal colony diameter was monitored from 3 to 7 days on a daily basis and was compared with that of the control fungi that had not been exposed to VOCs.

4.4. Effect of the Bacterial VOCs on the Synthesis of Mycotoxins. Toxigenic cultures of *A. flavus* CECT 2687 and *A. carbonarius* AC82 were exposed to *B. megaterium* BM344-1 volatiles as described in Section 2.2. At day 7 of co-incubation, three plugs of the fungal culture were removed with a cork-borer (7 mm). After weighing, OTA and AF were extracted in organic solvents as described by Ul Hassan *et al.*[39] The extracts were analyzed for mycotoxin content using HPLC.

4.5. Effect of BM344-1 VOCs on the Growth of *A. flavus* on Maize Kernels. Yellow maize kernels (Foody's, Thailand) were artificially infected with the toxigenic culture of *A. flavus* and exposed to *B. megaterium* BM344-1 VOCs to record their effect on fungal growth. For this purpose, kernels were purchased from the market, briefly sterilized in liquid bleach, and washed with sterilized distilled water. A loopful of fungal spores was taken from a 7 days-old colony of *A. flavus* in saline solution amended with Tween 80. A 10 μL (10^6 spores/mL) aliquot was spotted onto maize kernels. Infected kernels were placed in a Petri dish with nine holes (7 mm diameter) underneath to allow passage of bacterial volatiles emitted from a 24 h-old BM344-1 culture on TSA (placed at the bottom of a glass box). The lids were closed and completely sealed with Parafilm and incubated at 28 °C. Two *A. flavus*-inoculated controls were maintained, that is, kernels incubated in a glass box in the presence and absence of TSA agar plates (both in the absence of *B. megaterium* BM344-1).

The effect of BM344-1 volatiles on the growth of *A. flavus* was recorded as the fungal growth inhibition ratio (%) calculated by counting the number of maize kernels showing visible fungal growth at 7 dpi by using the formula as

$$\text{fungal growth inhibition ratio (\%)} = \frac{(C - T)}{C}100$$

C = Number of infected kernels in *A. flavus*-inoculated maize ear. T = Number of infected kernels in *A. flavus*-inoculated and BM344-1 volatiles-exposed maize ear.

4.6. Effect of BM344-1 Volatiles on AF Contamination on Maize Kernels. Maize ears were removed at the site of fungal inoculation from the treated kernels (Section 2.5) and thoroughly mixed. The AF contents of representative (2 g) samples were extracted in 10 mL of 70% methanol.[19] Enzyme-linked immunosorbent assay (ELISA) kits (RIDASCREEN Aflatoxin Total, Art no. R4701) obtained from R-Biopharm AG, Darmstadt, Germany, were used for AF analysis. An ELISA plate reader (Multiskan FC, Thermo Scientific, Waltham, MA, USA) installed with Skanlt software (Version 4.1. Thermo Scientific, MA, USA, 2015) was used to obtain the absorbance of ELISA plates wells. A calibration curve was generated by using absorbance data of known mycotoxins' standards solutions, and the absorbance values of unknown samples were added to the calibration curve to calculate the amount of toxins in our samples. For this purpose, the software Z9996 RIDA-SOFT Win (R-Biopharm, Darmstadt, Germany) was used.

4.7. Analysis of BM344-1 Volatile Bioactive Compounds. Bacterial volatiles were captured on activated charcoal (AC) and analyzed by GC−MS/MS as described by Ul Hassan *et al.*,[19] with little modification. Briefly, in 250 mL Erlenmeyer flasks, bacterial cell suspension was added to 100 mL of TSB media. Two valve rubber-corks were fitted to allow the passage of glass tubing. To the outer end of one tube, a volatile trap (glass Pasteur pipette filled with AC) was attached, while the other end was kept inside the flask at the neck level. The inner end of the second tube was placed ~1 cm above the TSB level, and the outer end was sealed with Parafilm. Flasks were incubated at 30 °C in a shaking incubator for 72 h. A gentle stream of nitrogen gas was introduced into the flask through the open end of the second tube for the removal of headspace volatiles to be trapped on AC. Captured volatiles on AC were eluted in dichloromethane and analyzed by GC with the set parameters as described Ul Hassan *et al.*[19] The mass spectral libraries of Wiley and NIST were used to compare the obtained spectra of unknown compounds. The control flasks were maintained with TSB without adding bacterial cells.

4.8. Statistical Analysis. The effect of bacterial VOCs on fungal development (colony size) *in vitro* and on maize kernels was presented as the fungal growth inhibition (%) as compared to that of unexposed fungi calculated by the formula given in Section 2.2. The mean values of mycotoxin synthesis inhibition in VOCs-exposed fungi were compared with that of the control using Student's "*t*-test". The data for mycotoxin synthesis inhibition on maize kernels was subject to ANOVA, followed by *post hoc* multiple comparison by Duncan's multiple range test at $p \leq 0.05$. Statistical software IBM SPSS (IBM SPSS Version 25 for macOS; SPSS Inc., Chicago, IL, USA) was used for these analyses.

AUTHOR INFORMATION

Corresponding Author

Samir Jaoua − *Department of Biological and Environmental Sciences, College of Arts and Science, Qatar University, Doha 2713, Qatar;* orcid.org/0000-0002-8819-131X; Phone: 00974 4403 4536; Email: samirjaoua@qu.edu.qa; Fax: 00974 4403 4531

Authors

Aya Ehab Saleh − *Department of Biological and Environmental Sciences, College of Arts and Science, Qatar University, Doha 2713, Qatar*

Zahoor Ul-Hassan − *Department of Biological and Environmental Sciences, College of Arts and Science, Qatar University, Doha 2713, Qatar*

Randa Zeidan − *Department of Biological and Environmental Sciences, College of Arts and Science, Qatar University, Doha 2713, Qatar*

Noora Al-Shamary − *Environmental Science Center, Qatar University, Doha 2713, Qatar*

Thoraya Al-Yafei − *Environmental Science Center, Qatar University, Doha 2713, Qatar*

Hajer Alnaimi − *Environmental Science Center, Qatar University, Doha 2713, Qatar*

Nayla Salah Higazy − *Department of Biological and Environmental Sciences, College of Arts and Science, Qatar University, Doha 2713, Qatar*

Quirico Migheli − *Dipartimento di Agraria and Desertification Research Centre (NRD), Università degli Studi di Sassari, Sassari I-07100, Italy*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c00816

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This project was made possible by the NPRP grant # 8-392-4-003 from the Qatar National Research Fund (a member of Qatar Foundation). The findings achieved herein are solely the responsibility of the authors.

REFERENCES

(1) Kensler, T. W.; Roebuck, B. D.; Wogan, G. N.; Groopman, J. D. Aflatoxin: A 50-Year Odyssey of mechanistic and translational toxicology. *Toxicol. Sci.* **2011**, *120*, S28−S48.

(2) Agriopoulou, S.; Stamatelopoulou, E.; Varzakas, T. Advances in occurrence, importance, and mycotoxin control strategies: prevention and detoxification in foods. *Foods* **2020**, *9*, 137.

(3) Rotimi, O. A.; Rotimi, S. O.; Goodrich, J. M.; Adelani, I. B.; Agbonihale, E.; Talabi, G. Time-course effects of acute aflatoxin B1 exposure on hepatic mitochondrial lipids and oxidative stress in rats. *Front. Pharmacol.* **2019**, *10*, 467.

(4) Ostry, V.; Malir, F.; Toman, J.; Grosse, Y. Mycotoxins as human carcinogens—The IARC monographs classification. *Mycotoxin Res.* **2017**, *33*, 65−73.

(5) Heussner, A.; Bingle, L. Comparative ochratoxin toxicity: A review of the available data. *Toxins* **2015**, *7*, 4253−4282.

(6) Ji, F.; He, D.; Olaniran, A. O.; Mokoena, M. P.; Xu, J.; Shi, J. Occurrence, toxicity, production and detection of Fusarium mycotoxin: A review. *Food Prod., Process. Nutr.* **2019**, *1*, 6.

(7) Omotayo, O. P.; Omotayo, A. O.; Mwanza, M.; Babalola, O. O. Prevalence of mycotoxins and their consequences on human health. *Toxicol. Res.* **2019**, *35*, 1−7.

(8) Phokane, S.; Flett, B. C.; Ncube, E.; Rheeder, J. P.; Rose, L. J. Agricultural practices and their potential role in mycotoxin contamination of maize and groundnut subsistence farming. *S. Afr. J. Sci.* **2019**, *115*, 1−6.

(9) Lagogianni, C. S.; Tsitsigiannis, D. I. Effective chemical management for prevention of aflatoxins in maize. *Phytopathol. Mediterr.* **2018**, *57*, 186−197.

(10) Carvalho, F. P. Pesticides, environment, and food safety. *Food Energy Secur.* **2017**, *6*, 48−60.

(11) Popiel, D.; Dawidziuk, A.; Koczyk, G.; Mackowiak, A.; Marcinkowska, K. Multiple facets of response to fungicides—the influence of azole treatment on expression of key mycotoxin biosynthetic genes and candidate resistance factors in the control of resistant Fusarium strains. *Eur. J. Plant Pathol.* **2017**, *147*, 773−785.

(12) Calado, T.; Venâncio, A.; Abrunhosa, L. Irradiation for mold and mycotoxin control: A Review. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13*, 1049−1061.

(13) Venkatesh, N.; Keller, N. P. Mycotoxins in conversation with bacteria and fungi. *Front. Microbiol.* **2019**, *10*, 403.

(14) Hathout, A. S.; Aly, S. E. Biological detoxification of mycotoxins: A review. *Ann. Microbiol.* **2014**, *64*, 905−919.

(15) Farbo, M. G.; Urgeghe, P. P.; Fiori, S.; Marcello, A.; Oggiano, S.; Balmas, V.; Ul Hassan, Z.; Jaoua, S.; Migheli, Q. Effect of yeast volatile organic compounds on ochratoxin A-producing *Aspergillus carbonarius* and *A. ochraceus*. *Int. J. Food Microbiol.* **2018**, *284*, 1−10.

(16) Tilocca, B.; Balmas, V.; Ul Hassan, Z.; Jaoua, S.; Migheli, Q. A proteomic investigation of *Aspergillus carbonarius* exposed to yeast volatilome or to its major component 2-phenylethanol reveals major shifts in fungal metabolism. *Int. J. Food Microbiol.* **2019**, *306*, 108265.

(17) Zeidan, R.; Ul-Hassan, Z.; Al-Thani, R.; Balmas, V.; Jaoua, S. Application of low-fermenting yeast *Lachancea thermotolerans* for the control of toxigenic fungi *Aspergillus parasiticus*, *Penicillium verrucosum* and *Fusarium graminearum* and their mycotoxins. *Toxins* **2018**, *10*, 242.

(18) Alasmar, R.; Ul-Hassan, Z.; Zeidan, R.; Al-Thani, R.; Al-Shamary, N.; Alnaimi, H.; Migheli, Q.; Jaoua, S. Isolation of a novel *Kluyveromyces marxianus* strain QKM-4 and evidence of its volatilome production and binding potentialities in the biocontrol of toxigenic fungi and their mycotoxins. *ACS Omega* **2020**, *5*, 17637−17645.

(19) Ul Hassan, Z.; Al Thani, R.; Alnaimi, H.; Migheli, Q.; Jaoua, S. Investigation and application of *Bacillus licheniformis* volatile compounds for the biological control of toxigenic *Aspergillus* and *Penicillium* spp. *ACS Omega* **2019**, *4*, 17186−17193.

(20) Zeidan, R.; Ul-Hassan, Z.; Al-Thani, R.; Migheli, Q.; Jaoua, S. *In-vitro* application of a Qatari *Burkholderia cepacia* strain (QBC03) in the biocontrol of mycotoxigenic fungi and in the reduction of ochratoxin A biosynthesis by *Aspergillus carbonarius*. *Toxins* **2019**, *11*, 700.

(21) Higazy, N. S.; Saleh, A. E.; Ul Hassan, Z.; Al Thani, R.; Migheli, Q.; Jaoua, S. Investigation and application of *Bacillus pumilus* QBP344-3 in the control of *Aspergillus carbonarius* and ochratoxin A contamination. *Food Control* **2021**, *119*, 107464.

(22) Werner, S.; Polle, A.; Brinkmann, N. Belowground communication: Impacts of volatile organic compounds (VOCs)

from soil fungi on other soil-inhabiting organisms. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 8651−8665.

(23) Tilocca, B.; Cao, A.; Migheli, Q. Scent of a Killer: Microbial volatilome and its role in the biological control of plant pathogens. *Front. Microbiol.* **2020**, *11*, 41.

(24) Schulz-Bohm, K.; Martín-Sánchez, L.; Garbeva, P. Microbial volatiles: small molecules with an important role in intra- and inter-kingdom interactions. *Front. Microbiol.* **2017**, *8*, 2484.

(25) Lazazzara, V.; Perazzolli, M.; Pertot, I.; Biasioli, F.; Puopolo, G.; Cappellin, L. Growth media affect the volatilome and antimicrobial activity against *Phytophthora infestans* in four *Lysobacter* type strains. *Microbiol. Res.* **2017**, *201*, 52−62.

(26) Bruce, A.; Stewart, D.; Verrall, S.; Wheatley, R. E. Effect of volatiles from bacteria and yeast on the growth and pigmentation of sapstain fungi. *Int. Biodeterior. Biodegrad.* **2003**, *51*, 101−108.

(27) Lima, S. L.; Colombo, A. L.; de Almeida Junior, J. N. Fungal cell wall: Emerging antifungals and drug resistance. *Front. Microbiol.* **2019**, *10*, 2573.

(28) Garcia-Rubio, R.; de Oliveira, H. C.; Rivera, J.; Trevijano-Contador, N. The fungal cell wall: *Candida, Cryptococcus,* and *Aspergillus* species. *Front. Microbiol.* **2020**, *10*, 2993.

(29) Chaves-López, C.; Serio, A.; Gianotti, A.; Sacchetti, G.; Ndagijimana, M.; Ciccarone, C.; Stellarini, A.; Corsetti, A.; Paparella, A. Diversity of food-borne *Bacillus* volatile compounds and influence on fungal growth. *J. Appl. Microbiol.* **2015**, *119*, 487−499.

(30) Mannaa, M.; Oh, J. Y.; Kim, K. D. Biocontrol activity of volatile-producing *Bacillus megaterium* and *Pseudomonas protegens* against *Aspergillus flavus* and aflatoxin production on stored rice grains. *Mycobiology* **2017**, *45*, 213−219.

(31) Mannaa, M.; Kim, K. D. Biocontrol activity of volatile-producing *Bacillus megaterium* and *Pseudomonas protegens* against *Aspergillus* and *Penicillium* spp. Predominant in stored rice grains: Study II. *Mycobiology* **2018**, *46*, 52−63.

(32) Wheatley, R. E. The consequences of volatile organic compound mediated bacterial and fungal interactions. *Antonie van Leeuwenhoek* **2002**, *81*, 357−364.

(33) Fiori, S.; Urgeghe, P. P.; Hammami, W.; Razzu, S.; Jaoua, S.; Migheli, Q. Biocontrol activity of four non- and low-fermenting yeast strains against *Aspergillus carbonarius* and their ability to remove ochratoxin A from grape juice. *Int. J. Food Microbiol.* **2014**, *189*, 45−50.

(34) Humphris, S. N.; Bruce, A.; Buultjens, E.; Wheatley, R. E. The effects of volatile microbial secondary metabolites on protein synthesis in Serpula lacrymans. *FEMS Microbiol. Lett.* **2002**, *210*, 215−219.

(35) Rajaofera, M. J. N.; Wang, Y.; Dahar, G. Y.; Jin, P.; Fan, L.; Xu, L.; Liu, W.; Miao, W. Volatile organic compounds of *Bacillus atrophaeus* HAB-5 inhibit the growth of Colletotrichum gloeosporioides. *Pestic. Biochem. Physiol.* **2019**, *156*, 170−176.

(36) Abubakar, M.; Majinda, R. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). *Medicines* **2016**, *3*, 3.

(37) Mohy El-Din, E. S.; Mohyeldin, M. Component analysis and antifungal activity of the compounds extracted from four brown seaweeds with different solvents at different seasons. *J. Ocean Univ. China* **2018**, *17*, 1178−1188.

(38) Kumar, R.; Kundu, A.; Dutta, A.; Saha, S. Profiling of volatile secondary metabolites of *Chaetomium globosum* for potential antifungal activity against soil borne fungi. *J. Pharmacogn. Phytochem.* **2020**, *9*, 922−9276.

(39) Ul Hassan, Z.; Al-Thani, R. F.; Migheli, Q.; Jaoua, S. Detection of toxigenic mycobiota and mycotoxins in cereal feed market. *Food Control* **2018**, *84*, 389−394.