Iranian Journal of Microbiology

Volume 17 Number 2 (April 2025) 303-311
DOI: http://doi.org/10.18502/ijm.v17i2.18397

ORIGINAL ARTICLE

Antifungal effect of soil *Bacillus* bacteria on pathogenic species of the fungal genera *Aspergillus* and *Trichophyton*

Mahnour Alsadat Taghavi[1], Maryam Ahmadi[1], Davoud Dehghan-Nayeri[1], Zahra Salehi[2], Masoomeh Shams-Ghahfarokhi[3], Fatemehsadat Jamzivar[2], Mehdi Razzaghi-Abyaneh[2*]

[1]Department of Microbiology, Faculty of Pharmacy, Islamic Azad University, Tehran, Iran
[2]Department of Mycology, Pasteur Institute of Iran, Tehran, Iran
[3]Departmentt of Mycology, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran

Received: October 2024**, Accepted:** February 2025

ABSTRACT

Background and Objectives: The increasing prevalence of fungal infections due to antifungal resistance underscores the need for novel treatment strategies. The present study aimed to investigate the inhibitory effects of soil-originated antagonistic bacteria against *Aspergillus* and *Trichophyton* species.

Materials and Methods: Fifty soil samples collected from Isfahan and Khuzestan provinces by using the Zig-Zag method were cultured on glucose-yeast extract (GY) agar around fungal colonies to isolate antagonistic bacteria. Antifungal activity was assessed by measuring clear zones around the colonies of *A. niger, A. fumigatus, T. rubrum,* and *T. mentagrophytes* by co-culture linear method. Potent antagonistic bacteria were identified by 16S rRNA sequencing, and evaluated for antifungal activity using disk diffusion assays compared with amphotericin B and ketoconazole.

Results: Among 50 samples, fifteen showed antifungal effects, yielding 55 bacterial strains. Four isolates with strong antifungal activity against all tested fungi were identified as *Bacillus subtilis, B. licheniformis, B. axarquiensis,* and *Bacillus* sp. These bacteria were distributed in distinct clusters phylogenitically and showed diverse antifungal activity.

Conclusion: The results suggest the potential of soil-derived *Bacillus* species as promising antifungal agents. Further studies are recommended to identify their inhibitory metabolites, their ability as biocontrol agents against soil habitated fungi and to explore their mechanism of action and spectrum of activity.

Keywords: Antifungal activity; *Aspergillus*; *Trichophyton*; *Bacillus*; Molecular identification; Soil bacteria

INTRODUCTION

The incidence of fungal infections has raised rapidly in past two decades, impacting 20 to 25% of the global population and resulting in 1.6 million fatalities every year (1). This is related to the rise in immunosuppressive diseases such as cancers and malignancies and acquired immunodeficiency syndrome. On the other hand, the widespread use of antifungal medications in treatment of fungal infections has contributed to the development of emerging resistance (2, 3). Furthermore, most existing medicines have several drawbacks, including toxicity, inadequate absorption, and inefficacy. Today's global mortality rate for fungal illnesses varies from 30% to 80%. As a result, the treatment of fungal infections

*Corresponding author: Mehdi Razzaghi-Abyaneh, Ph.D, Department of Mycology, Pasteur Institute of Iran, Tehran, Iran. Tel: +98-2164112123 Fax: +98-2164112125 Email: mrab442@pasteur.ac.ir

has become an important determinant of health (4).

Many efforts have been taken to find new sources of antifungal compounds (5). Soil microorganisms have been a significant source of antibiotics via their secondary metabolites. Antibiotic-producing bacteria serve as the permanent soil microflora, providing a source of many biologically active chemicals that are commonly exploited in the pharmaceutical sector (6). *Bacillus* species are considered as imperative sources of natural antimicrobial compounds (7). It has been shown that around 4-5% of the *Bacillus* genome is involved in producing biologically active components (8). The genus *Bacillus* comprises 266 species of bacteria with rod-shape and positive for Gram staining. Their diversity in physiological properties, ability to form endospores and biocontrol have contributed to their immense distribution in natural habitates including soil as the main reservoir, water, food, feed and animal gut microbiota (9).

The genus *Bacillus* comprises small mesophilic and neutrophilic vegetative cells with *B. subtilis, B. licheniformis, B. amyloliquefaciens* and *B. pumilus* being the main members. The ability of *B. subtilis* group to produce a wide array of beneficial metabolites with antimicrobial activity has been recognized for decades (9). Up to now, several various antifungal metabolites, including itorin, bacillomyxin, mycosubtilin, bacillopeptin, and phenjacin, have been reported in various *Bacillus* species (10). These molecules are mainly peptides with cyclic hydrophobic structures containing intramolecular thioether bonds and act as degrading enzymes with cellulase, protease, glucanase, and chitinase activities (11, 12). Therefore, *Bacillus* spp. are still a promising source for the identification of antifungal compounds. The present study aimed to isolate *Bacillus* species with inhibitory effect on pathogenic fungi species of *Aspergillus* and *Trichophyton*, from soil samples and to investigate the inhibition percentage of *Bacillus* metabolites compared to the antifungal drugs.

MATERIALS AND METHODS

Soil samples. In total, 50 samples of soils were gathered from 15 distinct geographical zones in Shahreza (Isfahan province) and Izeh (Khuzestan province). The soils were sampled using the zigzag technique at a 3-5 cm depth, then sieved and collected. The soils were kept in sterile zip bags at 4°C for further analysis (13).

Initial screening of soil samples with antifungal effect: Fungal and soil suspension preparation. The fungal strains, *Aspergillus fumigatus* ATCC5009, *Aspergillus niger* ATCC5010, *Trichophyton rubrum* ATCC613, and *Trichophyton mentagrophytes* ATCC5809, were obtained from the Pasteur Institute of Iran (http://fa1.pasteur.ac.ir/Pages.aspx?id=1152). The fungi were cultured on Sabouraud dextrose agar slants at 28°C for 7 days. Fungal spore suspensions were prepared by gentle rubbing the colony surface in presence of sterile distilled water contained Tween 80 (0.1%, v/v) and counted to adjust at a concentration of 2×10^5 spores/ml by Neubauer slide (14). An amount of 0.1 g of each soil sample was suspended in one ml sterile saline (0.9% NaCl) and the supernatant was separated after centrifuged at 2500 rpm for 30 seconds.

Determination of antagonistic soil samples. The ocular examination method was used to screen the soil samples with antifungal activity (15). In each plate of glucose-yeast extract (GY) agar (Glucose 5%, Yeast extract 1%, Agar 1.5%), a volume of 10 µL of fungal (*A. niger, A. fumigatus, T. rubrum*, and *T. mentagrophytes*) suspension (containing 200 spores/ml) were cultured at the center of plate and soil suspensions (10 µL) were cultured in a distance of 1.5 cm around fungal suspension at 28°C for 7-10 days. Soil samples that inhibited the fungal growth were chosen for further analyses.

Isolation of antagonistic bacteria from soil samples. A volume of 10 µL of soil suspensions which showed inhibitory activity on fungal growth were cultured on GY agar. After a three-day incubation at 28°C, every single colony was sub-cultured on a separate GY plate at 28°C for 3-5 days. Bacterial suspensions (with 1.5×10^8 bacteria/mL equal to 0.5 MacFarland) were prepared from all bacterial isolates and cultured around pre-cultured fungal colonies by co-culture linear method. Control cultures contained distilled water instead of bacteria cultures. After incubating for 7-10 days at 28°C, the growth rate of fungi around bacterial isolates was compared to the control plates. Bacterial isolates with moderate to strong antifungal effect were counted. Bacterial isolates which strongly inhibited the growth of *A. niger,*

A. fumigatus, T. rubrum, and *T. mentagrophytes* were selected for further analyses.

Molecular identification of antagonistic bacteria. The bacterial strains were cultured on tryptic soy agar for 24 h at 37°C. Then, bacterial colonies were dissolved in 800 µL lysis buffer. The genomic DNA was extracted using a genomic DNA extraction kit (Bio Basic, Canada) according to the manufacturer's instructions. The genus and species of the bacterial strains were identified using 16S rRNA sequencing. PCR amplification of the 16s rRNA region was conducted to identify and differentiate the bactria. The reaction mixture contained the extracted DNA (2 µL), Taq DNA Polymerase Master Mix White (12 µL), 0.3 µL of each the 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1525R (5′AAGGAGGTGWTCCARCC-3′) primers (14) and water to gain a final volume of 25 µL. The reaction mixture was initially denatured at 95°C for 5 min, followed by 35 cycles of denaturation for 45 sec at 94°C, annealing for 45 sec at 58°C, an extension for 60 sec at 72°C, and a final extension at 72°C for 7 minutes. The PCR products 16s rRNA region were sequenced by the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit.

DNA sequences were manually edited and compared with other relevant available sequences using the BLAST in the GenBank database. Several representatives of these sequences were selected for the reconstruction of phylogenetic relationships. Multiple alignments of sequences were conducted using MEGA7 software and maximum likelihood (ML) analyses were performed by using RAxML version 8.2 run on the CIPRES Science Gateway and optimized using the GTRCAT option (Miller et al., 2010). Bootstrap values for maximum likelihood were 1000 replicates and the output files of the phylogenetic programs were visualized using Dendroscope V.3.2.8. The ML BS values exceeding 70%, were shown on appropriate clades in the shape of ML BS. *Bacillus thuringiensis* was used as outgroup taxa in the tree.

Quantitation of the antifungal activity of *Bacillus* strains: Solid cultures analysis. The antifungal activity of each strain in solid media was evaluated using the linear culture method (16). A volume of 10 µL of fungal suspensions (*A. niger, A. fumigatus, T. rubrum,* and *T. mentagrophytes*, containing 10^3 spores/mL) were cultured linearly at center of GY plates with a 1.5 cm distance from the linear cultures of antagonistic bacteria (10 µL of bacterial suspensions with a concentration of 1.5×10^8 bacteria) at 28°C for 4-14 days. The fungal growth after exposure to bacterial isolates were measured and compared to non-treated control plates. Fungal growth inhibition was calculated using the following formula:

$$I = (C-T)/C \times 100$$

where C is the growth of non-treated control fungal mycelia, T is the growth of treated fungal mycelia, and I is the fungal growth inhibition.

Antifungal activity of bacterial metabolites in liquid cultures. To evaluate the inhibitory effect of bacterial strains in liquid cultures, each bacterium was cultured in Erlenmayer flasks containing 50 mL GY broth at 28°C with shaking (150 rpm) for 48 h. The culture supernatants obtained by centrifugation at 4,400 rpm for 30 minutes at 4°C were filtered using a 0.45 µm Millipore filter. The volumes of 2.5, 1.25, 0.625, 0.312, and 0.156 mL of bacterial supernatant were separately added to each well of a six-well microplate containing 5 ml/well GY broth (0.5% yeast extract and 2% glucose, 1 liter distilled water) in triplicate. A volume of 500 µL of fungal suspensions containing 106 spores of each *A. fumigatus, A. niger, T. rubrum,* and *T. mentagrophytes*) was added to each well of the plates seperately and the plates were incubated at 28°C for 7-10 days. To measure the dry weight of fungal mycelia as an index of fungal growth, the content of each well was passed through a paper filter. Separated fungal mycelia were oven-dried at 80°C for three hours and were weighed. The inhibition effect of bacterial metabolites was calculated by comparing the dry weight of fungi in treated groups compared to the non-treated control group (17).

Comparison of bacterial antifungal effects with antifungal drugs. The antifungal effect of bacterial metabolites was compared to ketoconazole and amphotericin B using the disk diffusion method. A volume of 100 µl from *A. fumigatus, A. niger, T. rubrum* and *T. mentagrophytes* suspensions (containing 10^6 spores/mL) was separately cultured on Müller-Hinton agar. The blank sterile discs immersed in 10 µL of bacterial supernatant as well as ketoconazole (10 µg) and amphotericin B (20 µg) standard disks (MAST Diagnostica GmbH, Germany) were placed in the center of separate plates and incubated for four

days at 28°C. Antifungal activity was measured by calculating clear zones of no fungal growth around the disks (18).

Statistical analysis. Statistical analysis was carried out using the one-way ANOVA test (Tukey range) and P values <0.05 were considered significant.

RESULTS

Soil samples and bacterial strains with antifungal activity. Out of 50 soil samples, fifteen samples inhibited the growth of *Aspergillus* and *Trichophyton* species. Fifty-five bacterial strains were identified, with 16 strains demonstrating moderate to high inhibitory activity against tested fungi. Among them, four isolates strongly inhibited growth of both *Aspergillus* and *Trichophyton* species.

Molecular identification of antagonistic bacteria. The 16S rRNA sequencing of four bacterial isolates with strong antifungal activity showed that they belonged to *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus axarquiensis* and *Bacillus* sp.. Their 16s rRNA sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/).

For reconstructing the 16S ribosomal RNA phylogenetic tree, sequences of four *Bacillus* species identified in our study were compared with the sequences of *Bacillus* species deposited in the GenBank (n=17) and an outgroup (n=1) (Fig. 1). The phylogenetic tree was divided into three clades I, II, and III. Clade I contained *Bacillus axarquiensis* strains, clade II included *Bacillus subtilis* strains, and clade III includes *Bacillus licheniformis* strains. Clade III consisted of two clusters which contained the *Bacillus* sp. identified in this study.

Antifungal activity measurement. The minimum fungal growth was recorded for *T. rubrum* against *B. subtilis*, *T. rubrum*, and *T. mentagrophytes* against *B. licheniformis*, and *T. mentagrophytes* against *Bacillus* sp. The highest growth diameters of inhibition were reported for *A. fumigatus* and *A. niger* (16.1 and 17.4 mm, respectively) in exposure to *B. licheniformis.* The minimum growth of *A. fumigatus* was seen against *Bacillus* sp. and *B. subtilis* (growth diameters of 8.3 and 8.8 mm, respectively). The minimum growth of *A. niger* was seen against *B. subtilis* (growth diameter of 6.4 mm) (Table 1 and Fig. 2).

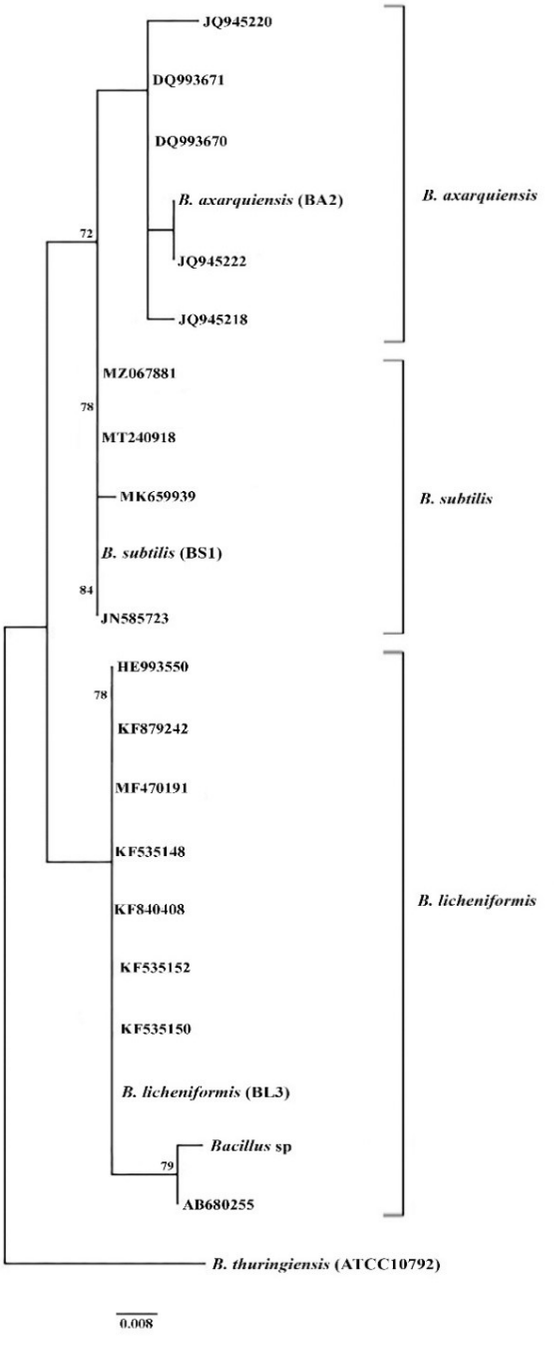

Fig. 1. The phylogenetic tree of 16S ribosomal RNA constructed by RAxML analysis from isolates of this study and isolates deposited in the GenBank. *Bacillus thuringiensis* was used as an outgroup to root the dendrogram. The bootstrap values greater than 70% are shown above the branches.

The dry weight of fungal mycelia increased by decreasing the proportion of bacterial supernatants (Table 2 and Fig. 2). *B. licheniformis* inhibited the growth of *T. rubrum*, *T. mentagrophytes*, *A. niger,* and

Table 1. The growth diameter (mm) of fungal colonies (*Aspergillus* and *Trichophyton* spp.) exposed to four *Bacillus* species in linear co-culture on GY agar.

Bacteria	*A. niger*	*A. fumigatus*	*T. rubrum*	*T. mentagrophytes*
B. subtilis	6.4	8.8	0	9
B. licheniformis	16.1	17.4	0	0
B. axarquiensis	15.11	9.6	4	10
Bacillus sp.	13.9	8.3	8	0
Non-treated control	40.2	28.4	20	30

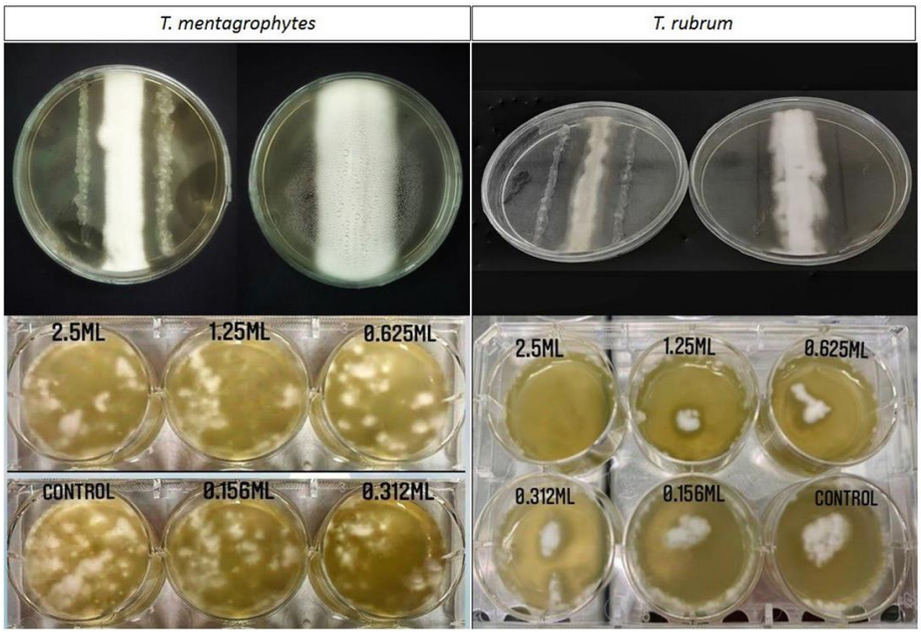

Fig. 2. The antifungal activity of *B. subtilis* on *T. rubrum* and *T. mentagrophytes* in linear co-culture assay (above) and liquid cultures in GY broth in 6-well microplates (below).

A. fumigatus by 93.6%, 85.7%, 70.2%, and 55.4%, respectively. The growth inhibition percentage of *B. subtilis* ranged from 79.9% (for *T. mentagrophytes*) to 95.5% (for *A. fumigatus*). *B. axarqueinsis* inhibited the growth of *Aspergillus* and *Trichophyton* species by 71.3% to 77.7%, accordingly (Table 2 and Fig. 3).

Comparison of bacterial inhibition effect to antifungal drugs. The lowest growth inhibition percentages were for *B. licheniformis* (19.1%) and *B. subtilis* (20%) against *A. niger* and *A. fumigatus*, respectively. The highest growth inhibition was seen for *Trichophyton* spp. by *B. icheniformis* (65%) and *B. subtilis* (60%). The highest inhibition percentages of *B. subtilis* against *A. niger* and *A. fumigatus* were 51.2% and 38.7%, respectively. The growth inhibition percentage of ketoconazole and amphotericin B ranged from 31.25% to 46.25% (Table 3 and Fig. 4).

DISCUSSION

Aspergillus and *Trichophyton* are among the most important fungal pathogens (19). *Aspergillus* can thrive in practically any substance or organic matter and is found in various habitats, including soil, air, and water. The most prevalent *Aspergillus* species identified from opportunistic fungal infections in humans and animals are *A. fumigatus* and *A. niger* (20). They are causatives of infections such as aspergilloma, allergic bronchopulmonary aspergillosis, chronic pulmonary aspergillosis, and invasive aspergillosis in immunosuppressed hosts (21, 22). *T. rubrum* is an anthropophilic fungus responsible

Table 2. Fungal growth inhibitory effects of *Bacillus* metabolites in liquid cultures on 6-well microplates by microbioassay.

Bacterial strains	Bacterial metabolites	*A. fumigatus*		*A. niger*		*T. rubrum*		*T. mentagrophytes*	
		Dry weight	Inhibition	Dry weight	Inhibition	Dry weight	Inhibition	Dry weight	Inhibition
	(ml)	(mg)	(%)	(mg)	(%)	(mg)	(%)	(mg)	(%)
B. subtilis	2.5	2.7	90.3	1.1	95.51	2.5	93.6	4.2	79.2
	1.25	8.2	71.17	7.9	67.75	12.1	69.0	6.3	68.9
	0.625	12.8	54.95	8.6	64.89	14.5	62.9	9.7	52.0
	0.312	16.8	41.12	14.0	42.85	21.5	45.0	12.1	40.3
	0.156	25.2	11.69	23.0	6.13	32.1	17.9	15.9	21.7
	0.0	25.8	0.0	24.5	0.0	39.1	0.0	20.3	0.0
B. licheniformis	2.5	7.0	55.41	6.7	70.22	1.9	93.6	3.0	85.7
	1.25	8.0	49.04	10.5	53.33	5.0	83.3	7.1	66.4
	0.625	8.6	45.22	13.4	40.44	9.4	68.5	9.0	53.7
	0.312	9.1	42.03	17.9	20.44	14.5	51.4	11.8	44.3
	0.156	10.5	33.12	19.2	14.66	22.1	26.1	13.4	37.0
	0.0	15.7	0.0	22.5	0.0	30.0	0.0	21.3	0.0
B. axarqueinsis	2.5	4.2	72.0	6.3	74.49	7.2	77.7	5.4	71.3
	1.25	8.6	68.84	6.9	72.06	8.3	74.7	6.4	65.9
	0.625	13.4	51.44	8.1	62.34	13.4	58.6	8.9	52.5
	0.312	15.0	45.65	9.3	58.70	25.7	20.4	11.5	39.0
	0.156	18.9	31.52	10.2	32.79	29.7	14.9	15.1	19.7
	0.0	27.6	0.0	24.7	0.0	32.4	0.0	18.9	0.0
Bacillus sp.	2.5	10.4	70.22	8.9	68.43	9.6	67.6	8.3	58.6
	1.25	16.4	55.33	9.8	65.95	15.2	48.9	10.2	46.9
	0.625	18.6	40.44	14.7	47.87	13.7	42.0	11.9	38.2
	0.312	21.3	20.44	15.8	43.97	19.8	33.6	13.7	29.0
	0.156	26.5	14.66	19.8	30.14	24.7	17.4	16.5	14.5
	0.0	32.1	0.0	28.2	0.0	29.9	0.0	19.3	0.0

for over 60% of all dermatophytosis and deep dermal infections (23). *T. mentagrophytes* is a significant causative of zoonotic fungal infections, which can transfer to humans, especially children and the elderly, from asymptomatic pets (24). Due to the increasing rate of individuals with problems of health conditions and antifungal resistance, their treatment has become concerning (25, 26). Therefore, in the presented study, we evaluated the antifungal activity of four *Bacillus* spp. against species of *Aspergillus* and *Trichophyton*.

In this study, the inhibitory potential of different *Bacillus* spp. varied from strain to strain. *B. subtilis* showed a more significant inhibitory effect on *A. niger* than *A. fumigatus. B. axarqueinsis* inhibited *A. fumigatus* more than *A. niger.* Based on our results, overall, the highest inhibitory effect of our four *Bacillus* strains was seen against *Trichophyton* spp. in both solid and liquid bacterial cultures and disk diffusion. The inhibition percentages of *B. licheniformis* and *B. subtilis* disks against both *Trichophyton* species were about three times higher than those of *A. fumigatus* and *A. niger.* However, the fungal inhibition percentage of *B. subtilis* was higher against *A. fumigatus* compared to *Trichophyton* in liquid cultures.

Interestingly, our four *Bacillus* strains showed satisfactory antifungal activity in solid and liquid assays. However, compared to ketoconazole and amphotericin B, only *B. subtilis* had a significantly higher inhibition percentage against all four *Aspergillus* and *Trichophyton* species. The reason can be referred to as the presence of multiple antifungal metabolites in *B. subtilis* supernatant compared to other *Bacillus* strains. It has been previously shown that *B. subtilis* can produce iturin A and surfactin, fengycin, and bacillomycin with antifungal activity (27, 28). The satisfactory antifungal activity of *B. subtilis*

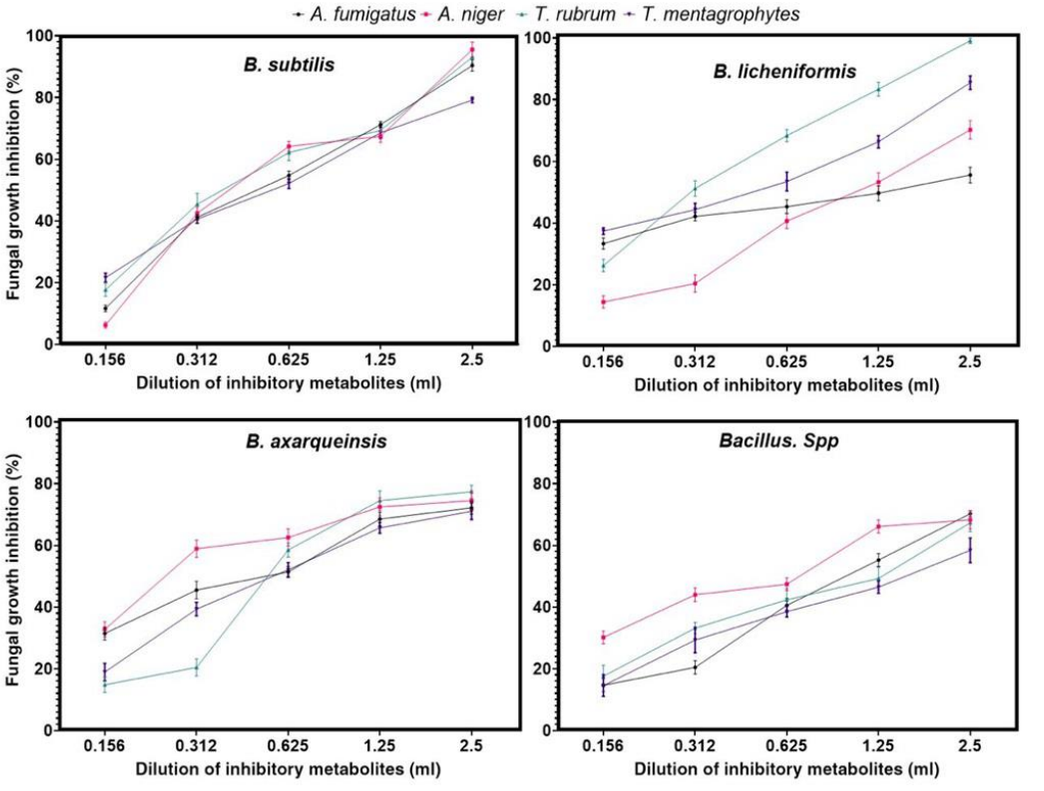

Fig. 3. The fungal growth inhibition percentages of different dilutions of bacterial metabolites against tested fungi of the genera *Aspergillus* and *Trichophyton*.

Table 3. Inhibition zones and percentage of fungal growth inhibition by different *Bacillus* species compared to ketoconazole and amphotericin B in disk diffusion assay

Treatments	*A. fumigatus*		*A. niger*		*T. rubrum*		*T. mentagrophytes*	
	Inhibition zone (mm)	Inhibition (%)	Inhibition zone (mm)	Inhibition (%)	Inhibition zone (mm)	Inhibition (%)	Inhibition zone (mm)	Inhibition (%)
B. subtilis	41	51.25	31	38.75	48	60	48	60
B. licheneformis	16	20	13	19.12	52	65	52	65
B. axarcoensis	28	35.4	22	27.5	43	53.7	43	53.7
Bacillus sp.	27	33.75	19	23.75	29	36.2	29	36.2
Ketoconazole	37	46.25	25	31.25	31	38.5	31	38.7
Amphotericin B	25	31.25	18	22.5	35	43.7	35	43.75

derivatives against *Microsporum canis*, *Candida albicans*, *Cryptococcus neoformans* was also reported by several studies (28-31). The *Bacillus* strains are likely to exert their antifungal activity through several potential mechanisms, including the production of enzymes that degrade fungal cell walls, secretion of antifungal metabolites such as surfactin or iturins, and competitive exclusion for essential nutrients (32).

CONCLUSION

Taken together, our study showed that *Bacillus* spp. could strongly inhibit the growth of *T. rubrum*, *T. mentagrophytes*, *A. niger*, and *A. fumigatus*. Further investigation to identify *Bacillus* metabolites responsible for antifungal activity, ability of identified bacilli as biocontrol agents against soil habitated fungi and to explore their spectrum of activity

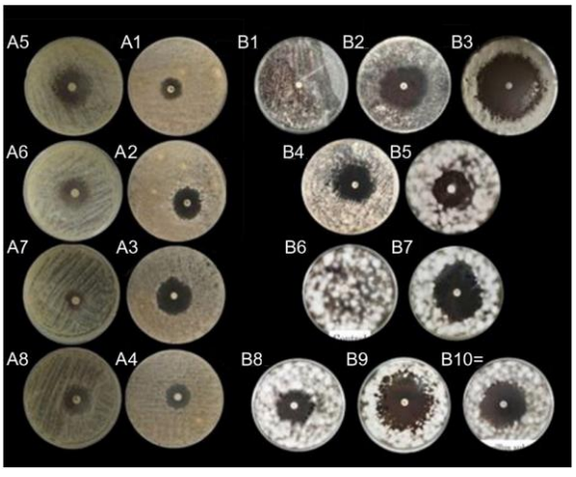

Fig. 4. A) The growth inhibition of *A. niger* by amphotericin B (A1), ketoconazole (A2), *B. axarquiensis* (A3), and *B. subtilis* (A4), disks and *A. fumigatus* inhibition by *B. axarquiensis* (A5) *B. subtilis* (A6), amphotericin B (A7), *B. Licheniformi* (A8) disks. B) The growth inhibition of *T. mentagrophytes* by ketoconazole (KTC) (B2), *B. subtilis* (B3), amphotericin B (AMP) (B4), *Bacillus* sp. (B5) disks compared to control (B1) and *T. rubrum* by amphotericin B (AMP) (B7), *Bacillus* sp. (B8), ketoconazole (KTC) (B9), and *B. subtilis* (B10) disks compared to control (B6).

and mode of action is recommended.

ACKNOWLEDGEMENTS

The authors are thankful of the personnel of the Mycology Department of the Pasteur Institute of Iran for helpful assistance in providing fascilities for fungal culture. This work was supported in part by the Pasteur Institute of Iran.

REFERENCES

1. Wang F, Zhang X, Chen G, Zhao Y. Living bacterial microneedles for fungal infection treatment. *Research (Wash D C)* 2020; 2020: 2760594.
2. Wiederhold NP. Antifungal resistance: current trends and future strategies to combat. *Infect Drug Resist* 2017; 10: 249-259.
3. Berman J, Krysan DJ. Drug resistance and tolerance in fungi. *Nat Rev Microbiol* 2020; 18: 319-331.
4. Beardsley J, Halliday CL, Chen SCA, Sorrell TC. Responding to the emergence of antifungal drug resistance: perspectives from the bench and the bedside. *Future Microbiol* 2018; 13: 1175-1191.
5. Vandeputte P, Ferrari S, Coste AT. Antifungal resistance and new strategies to control fungal infections. *Int J Microbiol* 2012; 2012: 713687.
6. Sharrar AM, Crits-Christoph A, Méheust R, Diamond S, Starr EP, Banfield JF, et al. Bacterial secondary metabolite biosynthetic potential in soil varies with phylum, depth, and vegetation type. *mBio* 2020; 11(3): e00416-20.
7. Amin M, Rakhisi Z, Zarei Ahmady A. Isolation and identification of *Bacillus* species from soil and evaluation of their antibacterial properties. *Avicenna J Clin Microbiol Infect* 2015; 2: 23233.
8. Stein T. *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Mol Microbiol* 2005; 56: 845-857.
9. Caulier S, Nannan C, Gillis A, Licciardi F, Bragard C, Mahillon J. Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Front Microbiol* 2019; 10: 302.
10. Dunlap CA, Bowman MJ, Rooney AP. Iturinic lipopeptide diversity in the *Bacillus subtilis* species group: important antifungals for plant disease biocontrol applications. *Front Microbiol* 2019; 10: 1749.
11. Caulier S, Gillis A, Colau G, Licciardi F, Liépin M, Desoignies N, et al. Versatile antagonistic activities of soil-borne *Bacillus* spp. and *Pseudomonas* spp. against Phytophthora infestans and other potato pathogens. *Front Microbiol* 2018; 9: 143.
12. Alamri SA. Enhancing the efficiency of the bioagent *Bacillus subtilis* JF419701 against soil-borne phytopathogens by increasing the productivity of fungal cell wall degrading enzymes. *Arch Phytopathol Plant Prot* 2014; 48: 159-170.
13. Jafari S, Aghaei SS, Afifi-Sabet H, Shams-Ghahfarokhi M, Jahanshiri Z, Gholami-Shabani M, et al. Exploration, antifungal and antiaflatoxigenic activity of halophilic bacteria communities from saline soils of Howze-Soltan playa in Iran. *Extremophiles* 2018; 22: 87-98.
14. Ranjbariyan A, Shams-Ghahfarokhi M, Kalantari S, Razzaghi-Abyaneh M. Molecular identification of antagonistic bacteria from Tehran soils and evaluation of their inhibitory activities toward pathogenic fungi. *Iran J Microbiol* 2011; 3: 140-156.
15. Shams-Ghahfarokhi M, Kalantari S, Razzaghi-Abyaneh M (2013). Terrestrial Bacteria from agricultural soils: versatile weapons against aflatoxigenic fungi. In: Razzaghi-Abyaneh M (ed) *Aflatoxins: Recent Advances and Future Prospects.* InTech, Rijeka.
16. Gebily DA, Ghanem GA, Ragab MM, Ali AM, Soliman NEK, El-Moity A, et al. Characterization and potential antifungal activities of three *Streptomyces* spp. as biocontrol agents against *Sclerotinia sclerotiorum*

infecting green beans. *Egypt J Biol Pest Control* 2021; 31: 1-15.
17. Mohammadi R, Abbaszadeh S, Sharifzadeh A, Sepandi M, Taghdir M, Youseftabar Miri N, et al. In vitro activity of encapsulated lactic acid bacteria on aflatoxin production and growth of *Aspergillus* spp. *Food Sci Nutr* 2021; 9: 1282-1288.
18. Bidaud A-L, Schwarz P, Herbreteau G, Dannaoui E. Techniques for the assessment of in vitro and in vivo antifungal combinations. *J Fungi (Basel)* 2021; 7: 113.
19. Paulussen C, Hallsworth JE, Álvarez-Pérez S, Nierman WC, Hamill PG, Blain D, et al. Ecology of aspergillosis: insights into the pathogenic potency of *Aspergillus fumigatus* and some other *Aspergillus* species. *Microb Biotechnol* 2017; 10: 296-322.
20. Boral H, Metin B, Döğen A, Seyedmousavi S, Ilkit M. Overview of selected virulence attributes in *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton rubrum*, and *Exophiala dermatitidis*. *Fungal Genet Biol* 2018; 111: 92-107.
21. Pinto E, Monteiro C, Maia M, Faria MA, Lopes V, Lameiras C, et al. *Aspergillus* species and antifungal susceptibility in clinical settings in the North of Portugal: cryptic species and emerging azoles resistance in *A. fumigatus*. *Front Microbiol* 2018; 9: 1656.
22. Gautier M, Normand AC, Ranque S. Previously unknown species of *Aspergillus*. *Clin Microbiol Infect* 2016; 22: 662-669.
23. Ciesielska A, Kawa A, Kanarek K, Soboń A, Szewczyk R. Metabolomic analysis of *Trichophyton rubrum* and *Microsporum canis* during keratin degradation. *Sci Rep* 2021; 11: 3959.
24. Gnat S, Łagowski D, Nowakiewicz A, Osińska M, Kopiński Ł. Population differentiation, antifungal susceptibility, and host range of *Trichophyton mentagrophytes* isolates causing recalcitrant infections in humans and animals. *Eur J Clin Microbiol Infect Dis* 2020; 39: 2099-2113.
25. Paiva Macedo JD, Dias VC. Antifungal resistance: why are we losing this battle? *Future Microbiol* 2024; 19: 1027-1040.
26. Gupta AK, Mann A, Polla Ravi S, Wang T. An update on antifungal resistance in dermatophytosis. *Expert Opin Pharmacother* 2024; 25: 511-519.
27. Kim PI, Ryu JW, Kim YH, Chi YT. Production of biosurfactant lipopeptides iturin A, fengycin, and surfactin A from *Bacillus subtilis* CMB32 for control of *Colletotrichum gloeosporioides*. *J Microbiol Biotechnol* 2010; 20: 138-145.
28. Ramachandran R, Shrivastava M, Narayanan NN, Thakur RL, Chakrabarti A, Roy U. Evaluation of antifungal efficacy of three new cyclic lipopeptides of the class bacillomycin from *Bacillus subtilis* RLID 12.1. *Antimicrob Agents Chemother* 2017; 62(1): e01457-17.
29. Tunsagool P, Ploypetch S, Jaresitthikunchai J, Roytrakul S, Choowongkomon K, Rattanasrisomporn J. Efficacy of cyclic lipopeptides obtained from *Bacillus subtilis* to inhibit the growth of *Microsporum canis* isolated from cats. *Heliyon* 2021; 7(9): e07980.
30. Lei S, Zhao H, Pang B, Qu R, Lian Z, Jiang C, et al. Capability of iturin from *Bacillus subtilis* to inhibit *Candida albicans* in vitro and in vivo. *Appl Microbiol Biotechnol* 2019; 103: 4377-4392.
31. Subramenium GA, Swetha TK, Iyer PM, Balamurugan K, Pandian SK. 5-hydroxymethyl-2-furaldehyde from marine bacterium *Bacillus subtilis* inhibits biofilm and virulence of *Candida albicans*. *Microbiol Res* 2018; 207: 19-32.
32. Bais HP, Fall R, Vivanco JM. Biocontrol of *Bacillus subtilis* against infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiol* 2004; 134: 307-319.