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# Identification and Study of Growth and Morphology of Phytopathogenic Fungi Under Effect of *Bacillus Subtilis* Culture, Isolated from Rizo and Planosphera of Some Medicinal Plants of Central Kazakhstan

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***Abstract:*** **In this paper the results of 16S rRNA and ITS region genotyping of the nucleotide sequences of *Bacillus subtilis* and some isolates of pathogenic fungi, as well as the results of studies of growth and morphology of pathogenic fungi under the influence of *Bacillus subtilis* (Ehrenberg) Cohn culture, held in the framework of the project 01 "Development of a biological product with growth-stimulating and fungicidal activity on the basis of rizo- and planosphera microflora and medicinal plants" which is part of Target scientific and technical program O.0590 "Creating, maintaining, keeping and use of microbial resources in the Republic of Kazakhstan" for 2012-2014.**

**Five isolates of pathogenic fungi were isolated from rizo- and planosphera of the wormwood (*Artemisia glabella Kar. Et Kir.*) and Ajania shrub (*Ajania fruticulosa (Ledeb.) Poljak*) in the Karaganda region of Kazakhstan. The strain of bacteria *Bacillus subtilis* was isolated from planosphera of Ajania and deposited in the Republican Collection of Microorganisms for the purpose of storage and warranty of Patent Procedure under number RKM B-0561.**

**The identity of the obtained 16S rRNA gene and ITS region nucleotide sequences of some isolates proved by comparing the nucleotide sequences loaded in the international database GenBank NCBI BLAST. Under the influence of the strain *Bacillus subtilis* RKM B-0561, the growth and development of the studied pathogenic fungi are inhibited to varying degrees depending on their species.**

**The strain of *Bacillus subtilis* RKM B-0561could be used to develop a new kind of biological products for controlling of plant pathogens.**

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***Keywords:*** **Bacillus Subtilis, Phytopathogenic Fungi, Culture-Morphological Characteristics, The Nucleotide Sequence, Antagonistic Activity**

## 1. INTRODUCTION

An important step in disease control programmes involves selection of effective biocontrol agents. *Bacillus* species including *B. subtilis* are known for their antifungal properties, hence their importance in the biological control of a number of plant and animal diseases [1] - [4].

An efficient antagonistic strain of *B. subtilis*, originally isolated from the rhizosphere of established tea bushes, was found to cause structural deformities in six pathogenic fungi under in vitro culture conditions. The inhibitory effect caused by volatiles was greater than that by diffusible compounds [5].

Based on a long term study on the rhizosphere microbiology of tea, involving a number of tea gardens of Indian Himalayan region, several strains of *Bacillus sp.* were isolated and screened for their antifungal activity against a range of saprophytic and pathogenic fungi [6], [7]. This investigation characterises one of the selected strains of *B. subtilis* [7] with respect to antagonistic activity and induction of structural abnormalities caused by production of diffusible and volatile compounds, in a number of fungi in culture.

Biocontrol appears to be a reliable alternative to chemical fungicides, which have raised serious concerns of food contamination, environmental pollution [8], and botanic phytotoxicity [9]. Since then, people have made considerable efforts to find different biocontrol methods for soil-borne diseases, including the application of plant extracts [10], antagonistic microorganisms [11], fungicides from antagonistic strains of fungi and bacteria [12], and disease-resistant varieties [13].

The results of the nucleotide sequence of microorganisms confirm classical identification methods [14], [15].

## 2. MATERIALS AND METHODS

*Research objects:* We used five isolates of pathogenic fungi from the current collection, consisting of 309 isolates of different taxonomic groups, as well as *Bacillus*

*subtilis* RKM B-0561 strain, isolated from wormwood and Ajania rizo- and planosphera.

*Species affiliation of the isolates by sequencing of 16S rRNA gene:* Preliminary identification of individual bacterial isolates was obtained by classical tests [16], [17]. Such identification included the shape of cells, Gram stain and colony morphology on solid nutrient media, liquid media, and biochemical tests. Genetic identification of isolates was performed by determining nucleotide sequences of 16S rRNA genes. Extraction and purification of DNA from bacterial cells were carried out by the hexadecyltrimethyl ammonium bromide (CTAB) method [18]. Fragments of the 16S rRNA gene were amplified using universal oligonucleotides FD1 (AGAGTTTGATC CTGGCTCAG) and RD1 (AAGGAGGTGATCCAGCC) as forward and reverse primers, respectively [19]. PCR was performed in a total volume of 50 µL of the reaction mixture containing approximately 10 ng of genomic DNA, 5x GoTaq reaction buffer, 0.2 mM of each deoxynucleoside triphosphate, 2 mM of each primer (fD1 and rD1) and 1.5 units of GoTaq DNA polymerase (Promega). Thermal cycling consisted of an initial denaturation at 95 °C in 5 min followed by 30 cycles of 1 min at 95 °C, 1 min at 50 °C, 2 min at 72 °C, and a final extension in 7 min at 72 °C. The PCR obtained products were purified with illustra GFX PCR DNA and gel band purification kit (Amersham Biosciences, GE Healthcare) according to the manufacturer's protocol. Partial 16S rRNA gene sequences were performed using a set of BigDye Terminanor v 3.1 Cycle sequencing Kit on an automated genetic analyzer ABI 3730xl (Applied Biosystems, USA) and the sequencing primers fD1 and rD1 according to the protocol of the manufacturer. The resulting nucleotide sequence was compared to all bacterial sequences available in the Gene Bank database using the BLAST program [20].

*Genome DNA isolation and ITS-region sequencing:* Preliminary identification of five pathogenic fungi species was carried out on the basis of physiological, morphological and cultural features study of the isolates [15], [21], [22].

DNA was extracted using the method of Kate Wilson (Wilson, 1997) [18].

For genetic identification of different fungal isolates the fungus-specific PCR-forward primer ITS 1 and the reverse primer ITS 4 (Sigma) were used and DNA amplification is done by using a thermal iCycler IQ5 (BioRad, USA). The sequencing of the amplified DNA fragments was carried out using of BigDye® Terminator v3.1 Ready Reaction Cycle Sequencing Kit on the ABI PRISM 3730 DNA analyzer (Applied Biosystems Inc., USA) according to the manufacturer's instructions. The nucleotide sequence was determined for approximately 560 base region of the ITS and 5.8S *r*DNA gene from each of fungal isolates [23].

*Antagonistic activity:* The culture of test bacteria was inoculated by solid "grass" on the surface of meat-peptone agar in a Petri dish and incubated at +37°C for 1-2 days for the accumulation of inhibitory compounds in the agar. Then agar blocks with grown culture of the strain were excised and installed on the surface of the Capek agar medium in another Petri dish pre-seeded with solid "lawn" of test cultures. Plates were incubated in an incubator at 28°C for 5-7 days [5], [16], [24]. The degree of the test culture new strain antagonist activity was judged by the width of test cultures growth inhibition area around the agar blocks (Fig. 2).

As the control of phytopathogenic fungi species they were planted in Petri dishes.

Micrographs of the pathogenic fungi hyphae-substrate and aerial mycelium were performed in the study of preparations obtained by the pressure drop [25], using a "Polivar" microscope with Nomarski interference optics.

## 3. Results and Discussion

The strain *Bacillus subtilis* RKM B-0561 is characterized by the following cultural-morphological features.

When culturing the strain on the meat-peptone agar at 37°C over 18-24 hours flesh-colored colonies are formed of oval or round shape, 5-6 mm in size, with wavy edges, finely wrinkled and mucous consistency. The growth in meat-peptone broth results in turbidity, the film formation on the medium surface and a sediment appearance on the bottom.

In the culture smears gram-positive rod-shaped cells with rounded or "chopped off" ends were revealed, arranged singly, in pairs, chains or crowded. Aerobic cells form endospores.

According to the results of genotyping 16S rRNA strain the following nucleotide sequence was obtained:

AACACGTGGGTAACCTGCCTGTAAGACTGGGAT
AACTCCGGGAAACCGGGGCTAATACCGGATGGTT
GTTTGAACCGCATGGTTCAGACATAAAAGGTGGC
TTCGGCTACCACTTACAGATGGACCCGCGGCGCA
TTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGC
GACGATGCGTAGCCGACCTGAGAGGGTGATCGGC
CACACTGGGACTGAGACACGGCCCAGACTCCTAC
GGGAGGCAGCAGTAGGGAATCTTCCGCAATGGAC
GAAAGTCTGACGGAGCAACGCCGCGTGAGTGATG
AAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGG
AAGAACAAGTGCCGTTCAAATAGGGCGGCACCTT
GACGGTACCTAACCAGAAAGCCACGGCTAACTAC
GTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAG
CGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGC
AGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCG
GCTCAACCGGGGAGGGTCATTGGAAACTGGGGA
ACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACG
TGTAGCGGTGAAATGCGTAGAGATGTGGAGGAAC
ACCAGTGGCGAAAGGCGACTCTCTGGTCTGTAAC
TGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC
AGGATTA

The identity of the sequence in comparison with the nucleotide sequences loaded in the GenBank NCBI BLAST international database (CP009749.1 *Bacillus subtilis*, CP009611.1 *Bacillus subtilis*, KM052377.1

*Bacillus subtilis*, KJ655543.1 *Bacillus subtilis*, KJ655538.1 *Bacillus subtilis*) is 99% at E value = 0.0.

Five isolates of pathogenic fungi characterized by the following cultural-morphological features.

*Alternaria alternata* 52 colonies on Czapek medium are thick, felted, grayish-brown, edge of the colony is lighter (Fig. 1 A). The reverse is not painted.

Microscopy revealed septate, not colored hyphae of different diameters. Diameter of thin hyphae is 1,91-2,87 micrometeres, thick - 3,83-5,75 m. Conidiophores are single, brown. Conidia are colored, dark brown, connected in straight-line (rare - branching) chains, mural, containing 3-7 transverse septa and 1 (rarely 2) incomplete longitudinal bulkhead. Dimensions of conidia are 28,73-51,72 x 9,58-13,41 m. Septa are constricted. The apical cell is short or long, a colorless sprout-like unit (Fig. 1 B).

According to the results of ITS region of isolates genotyping the following nucleotide sequence was obtained:

GATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAGTGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCG
CCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGC
GTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGG
CGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTA
AAGTAATTGGCAcGCCGGCTACTGGTTTCGGAGC
GCAGCACAAGTCGCACTCTTCATCTAGCAAAGGT
CTAGCATCCATTAAGCCTTTTTCAACTTTGACTCG
ATCAGGTA

The identity of the sequence in comparison with the nucleotide sequences loaded in the GenBank NCBI BLAST international database (HM222961.1 *Alternaria alternata*) is 99% at E value = 0.0.

Colonies of *Cladosporium cladosporioides* 11-4, on a potato agar medium are dark green, brownish, zoned, with a white edge, velvety in appearance (Fig. 1 C). Reverse is black with a white stripe on the edge. Smell is unpleasant.

Microscopy revealed the formation of conidial structures - single, erect, dichotomously branched, painted conidiophores. Basal conidia are elongated. Their dimensions are 13,06-8,126 x 4,08-3,27 m. Conidia are connected in long chains, greenish, unicellular, ellipsoidal or lemon-shaped, dimensions are 4,08-6,53 x 3,27 m (Fig. 1 D).

According to the results of ITS region of isolates genotyping the following nucleotide sequence was obtained:

TTACAAGTTGACCCCGGCCTCGGGCCGGGATGT
TCACAACCCTTTGTTGTCCGACTCTGTTGCCTCCG
GGGCGACCTGCCTCCGGGCGGGGGCCCCGGGTGG
ACATTTCAAACTCTTGCGTAACTTTGCAGTCTGAG
TAAATTTAATTAATAAATTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAATGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCG
CCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAG
CGTCATTTCACCACTCAAGCCTCGCTTGGTATTGG
GCGACGCGGTCCGCCGCGCGCCTCAAATCGACCG
GCTGGGTCTTTCGTCCCCTCAGCGTTGTGGAAACT
ATTCGCTAAAGGGTAGCAGCGGGAGGCCACGCCG
TAAAAC

The identity of the sequence in comparison with the nucleotide sequences loaded in the GenBank NCBI BLAST international database (JX868638.1 *Cladosporium cladosporioides*) is 99% at E value = 0.0.

Colonia *Cochliobolus tuberculatus* 1-3F on Czapek medium, taking a half of the dish square, is brownish-gray, pressed, the center is gray and fluffy, with a whitish band on the edge (Fig. 1 E). Reverse is black, lighter on the edge. Smell is the faint, unpleasant.

Microscopy revealed hyphae of different diameters (from 1,45 to 5,83 micrometeres) and color (more mature painted). Conidiophores are single, light brown, 4,37-5,83 m in diameter. Conidia are tetrachoric, dark brown, sometimes slightly bent, one cell more than the rest. Dimensions of conidia are 29,15-38,63 x 11,66 m (Fig. 1 F). Clearly visible oil droplets are into conidia young cells.

Colonies of *Fusarium sp*. 46, on the potato agar medium are whitish, fluffy, slightly zoned, occupying almost the entire cup. The center of the colony is pink (Fig. 1 G). Reverse is yellow, pink in center.

Microscopy revealed unpainted hyphae with granular contents, with a diameter of 19,23 to 5,77 micrometeres, with an intense and incorrect branching, resulting in formation of small structures such as unpainted microsclerotia. Some hyphae are densely packed in the strands. Macroconidia are absent, there is a small number of oval microconidia.

Because of the absence of macroconidia sporulation or other structures used for the identification, the species-level identification of the sample is not possible. Judging by the presence of microconidia and featured mycelium, the sample may belong to the genus *Fusarium* (Fig. 1 H).

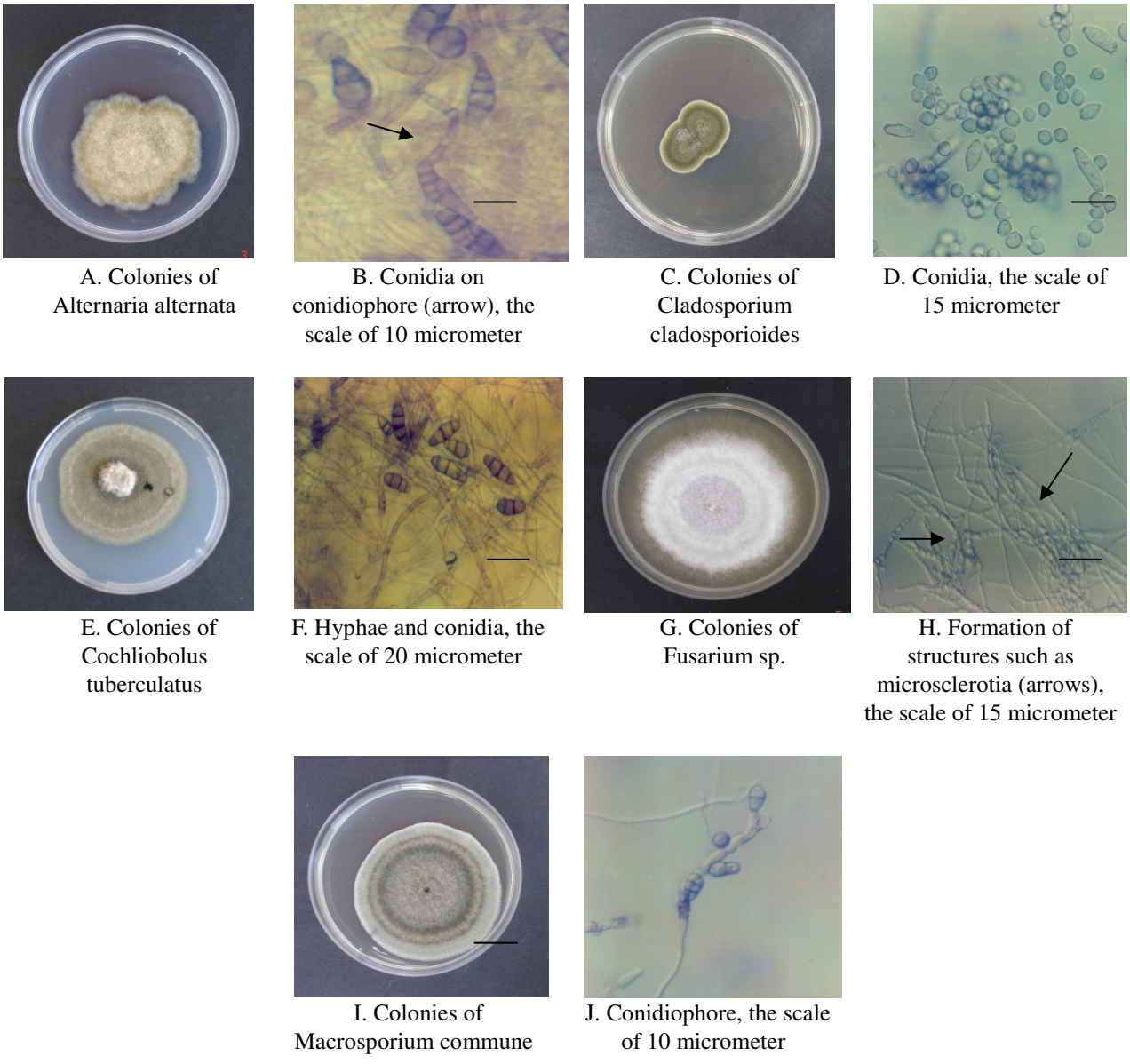
According to the results of ITS region of isolates genotyping the following nucleotide sequence was obtained:

CGTTGGGACCAGCGGAGGATCATTACCGAGTTT
ACAACTCCCAAACCCCTGTGAACATACCACTTGT
TGCTCGGCGGATCAGCCCGCTCCCGGTAAAACGG
GACGGCCCGCCAGAGGACCCCTAAACTCTGTTTC
TATATGTAACTTCTGAGTAAAACCATAAATAAAT
CAAAACTTTCAACAACGGATCTCTTGGTTCTGGC
ATCGATGAAGAACGCAGCAAAATGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAATC
TTTGAACGCACATTGCGCCCGCCAGTATTCTGGC
GGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA
AGCACAGCTTGGTGTTGGGACTCGCGTTAATTCG
CGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTT
CCATAGCGTAGTAGTAAAACCCTCGTTACTGRTA
ADCGTCGCGGCCACGCCGTTAAACCCCAACTTCT
GAATGTTGACCTCGGATCAGGTAGGAATACC

The identity of the sequence in comparison with the nucleotide sequences loaded in the GenBank NCBI BLAST international database (HQ658965.1 *Fusarium oxysporum* f. sp. cepae, FR852561.1 *Fusarium oxysporum* f. sp. melonis, HM756256.1 *Fusarium oxysporum* f. sp. phaseoli) is 99% at E value = 0.0.

Colonies of *Macrosporium commune* 3 on the potato agar medium are fluffy, gray-dirty-green, zoned (Fig. 1 I). Reverse is black, alternating with lighter areas.

Microscopy revealed septated hyphae of different diameters from 1,93 to 5,79 micrometeres. Mature hyphae became colored. Conidiophores are single, brown, long (102,32-133,20 micrometeres) (Fig. 1 J). Conidia are colored, dark brown, smooth or rough, collected in chains, mural, contain 4-6 transverse septa with constrictions and 0-1 (rarely 2) incomplete longitudinal bulkhead. Dimensions of the conidia are 23,17-34,74 x 9,65-11,58 m. In some conidia cells visible lipid droplets are located.



A. Colonies of Alternaria alternata

B. Conidia on conidiophore (arrow), the scale of 10 micrometer

C. Colonies of Cladosporium cladosporioides

D. Conidia, the scale of 15 micrometer

E. Colonies of Cochliobolus tuberculatus

F. Hyphae and conidia, the scale of 20 micrometer

G. Colonies of Fusarium sp.

H. Formation of structures such as microsclerotia (arrows), the scale of 15 micrometer

I. Colonies of Macrosporium commune

J. Conidiophore, the scale of 10 micrometer

Fig. 1 – Appearance of pathogenic fungi colonies on a Petri dish and their morphology

*Bacillus subtilis* RKM B-0561 showed a relatively low degree of antagonist activity (Table 1) with respect to the isolates of *Macrosporium commune* and *Fusarium sp.* The zone of inhibition was (21,8 ± 1,07) mm and (27,4 ± 1,78) mm, respectively. The degree of activity of that strain in relation to other isolates was slightly higher (Table I, Fig. 2).

It should be noted that the degree of antagonistic effect on development of studied phytopathogenic fungi decreases in the following order: *Cochliobolus tuberculatus*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Fusarium sp.*, *Macrosporium commune*. The most appreciable effect of *Bacillus subtilis* on the growth observed against fungus *Cochliobolus tuberculatus* 1-3F, in which the zone of inhibition was (34,2 ± 0,58) mm.

Table I: Inhibition zone of the pathogenic fungi growth when cultured with Bacillus subtilis RKM B-0561 on the 7th day (mm)

| № | Test-objects | Growth inhibition zone |
|---|---|---|
| 1 | *Alternaria alternata* (Fr.) Keissl.(=*Alternaria fasciculata* (Cooke et Ellis) Jones et Crout.), isolate 52, isolated from Artemisia | 32,8±1,24 |
| 2 | *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (=*Hormodendron cladosporioides* (Fresen.) Sacc., isolate 11-4, isolated from Ajania | 30,8±0,49 |
| 3 | *Cochliobolus tuberculatus* Sivan. (=*Curvularia tuberculata* B.L.Jain), isolate 1-3F, isolated from Artemisia | 34,2±0,58 |
| 4 | *Fusarium sp.*, isolate 46, isolated from Ajania | 27,4±1,78 |
| 5 | *Macrosporium commune* Rabenh., isolate 3, isolated from Artemisia | 21,8±1,07 |

n=3, P<0,002

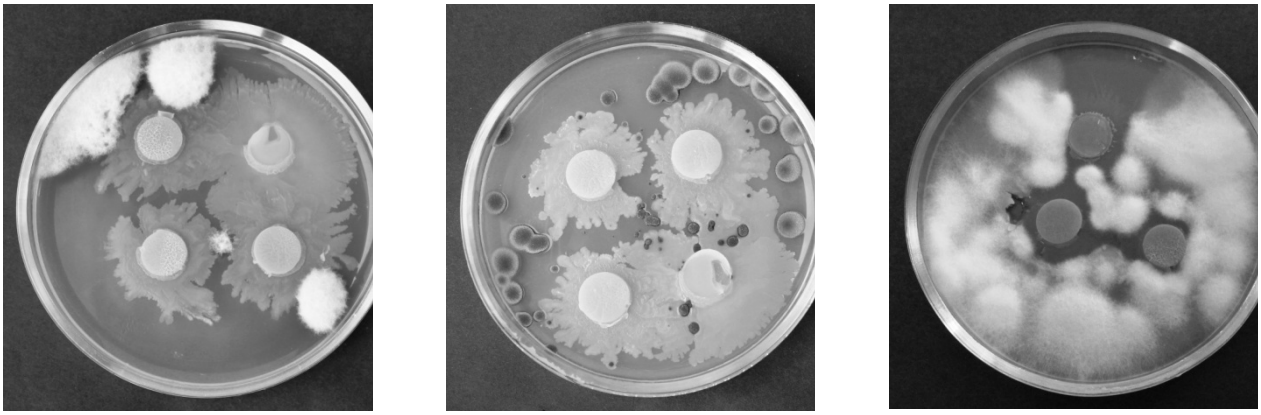


Fig. 2 – The joint growth of Bacillus subtilis with phytopathogenic fungi: Alternaria alternata, Cladosporium cladosporioides, Fusarium sp.

In the study of the influence of *Bacillus subtilis* culture on the growth and development of the fungus Alternaria alternata the appearance of nodules on hyphae was noted in submerged mycelium. Individual cells of hyphae become swollen and acquire a darker color (Fig. 3). Sometimes in these cells intragif development is observed, which in turn can thicken (Fig. 4).

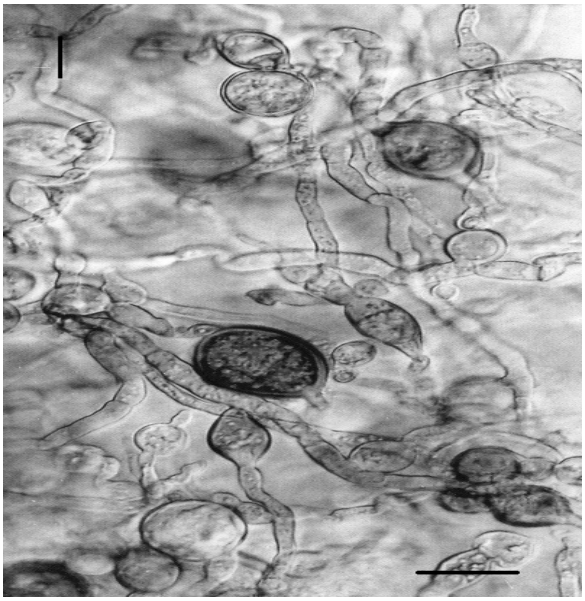


Fig. 3 – Thickening at the submerged Alternaria alternata hyphae, the scale of 10 micrometer

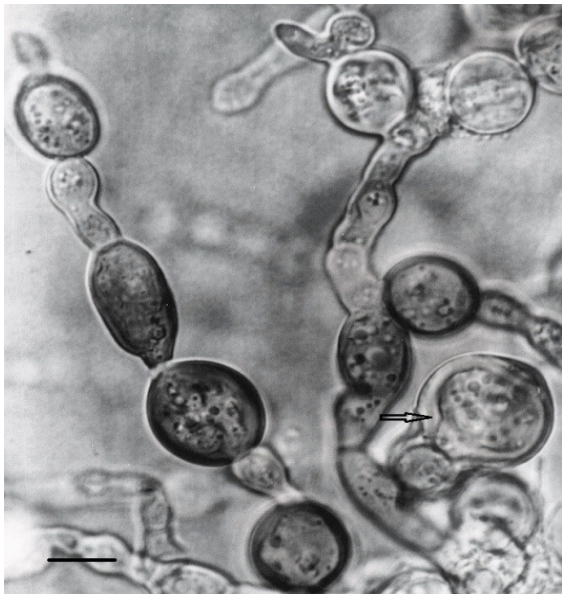


Fig. 4 – Thickening and intrahyphae hyphae (arrow) on the submerged hyphae of Alternaria alternata, the scale of 10 micrometer

In the control group Microscopy of *Alternaria alternata* aerial mycelium revealed hyphae of different diameters. Diameter of thin hyphae is 1,91-2,87 micrometeres, thick – 3,83-5,75 m.

Under the influence of *Bacillus subtilis* culture aerial hyphae of *Alternaria alternata* gather in bands of several

pieces (Fig. 5), the individual cells of hyphae grow in size and swell (Fig. 5, 6). Developing in them intrahyphae are seen quite clearly (Fig. 7).

Despite the rather significant changes under the influence of *Bacillus subtilis*, fungal hyphae cells contents are clearly visible, some hyphae and cells are highly vacuolated.

Colonies of *Alternaria alternata* are white, without characteristic color (in the control - thick, felted, grayish-brown, with lighter edge) and sporulation (Fig. 1 A).

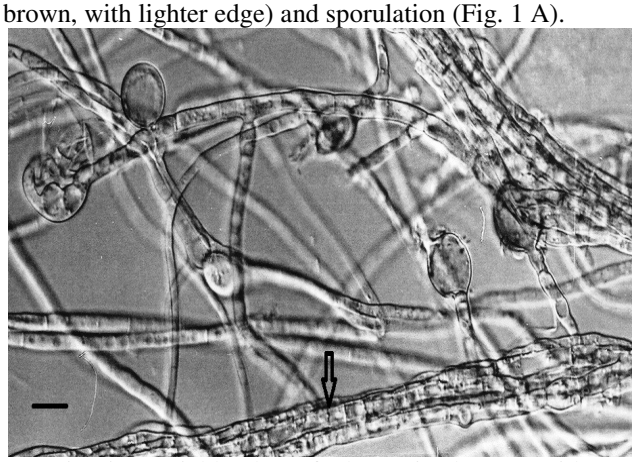


Fig. 5 – Aerial hyphae of Alternaria alternata with swellings and strands (arrow). Scale of 6 micrometer

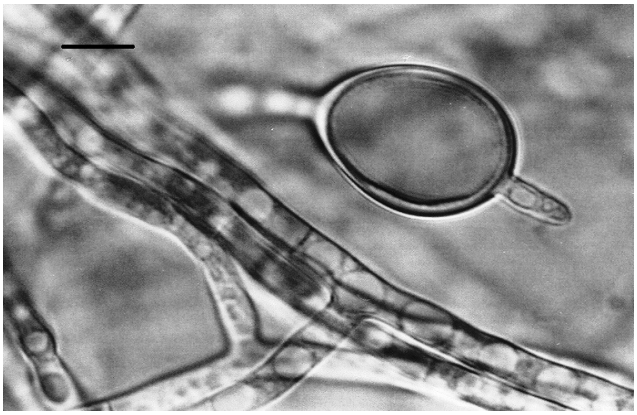


Fig. 6 – Aerial hyphae Alternaria alternata with swellings. Scale of 5 micrometer

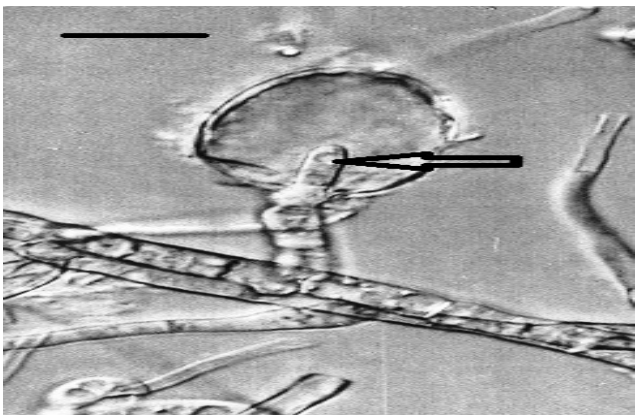


Fig. 7 – Intrahyphae (arrow) in the hyphae of the Alternaria alternata aerial mycelium. Scale of 6 micrometer

Substrate mycelium of *Cladosporium cladosporioides* (Fresen.) GA de Vries (isolate 11-4) under the influence of culture *Bacillus subtilis* is modified. Cells of the most hyphae swell, change their usual form and are dark colored (Fig. 8, 9).

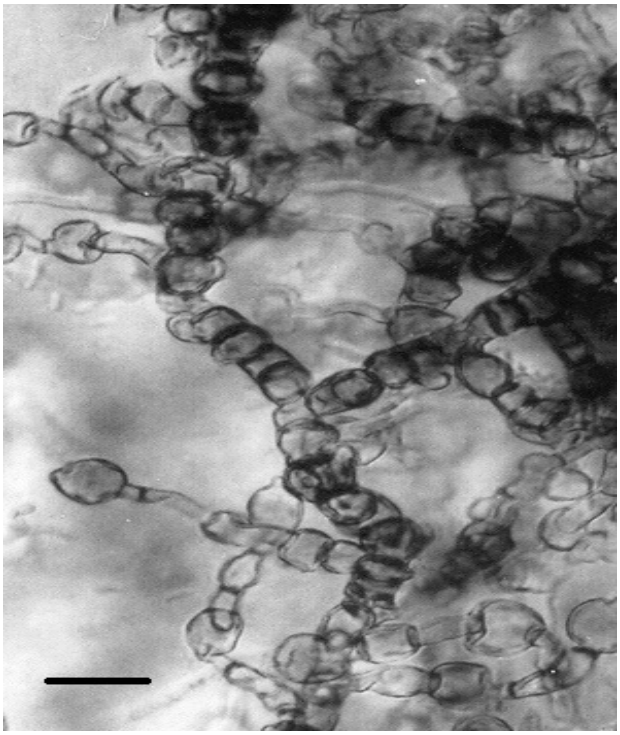


Fig. 8 – Hyphae of the Cladosporium cladosporioides substrate mycelium with swollen cells. Scale of 20 micrometer

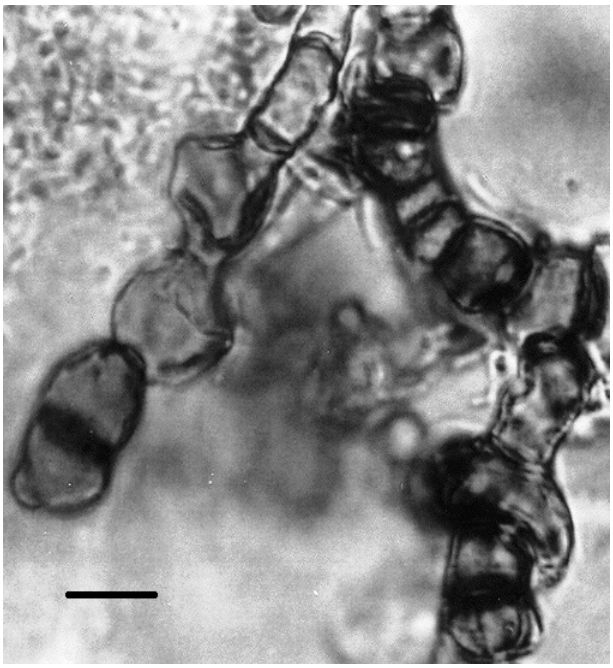


Fig. 9 – Swollen hyphal cells of Cladosporium cladosporioides substrate mycelium. Scale of 10 micrometer

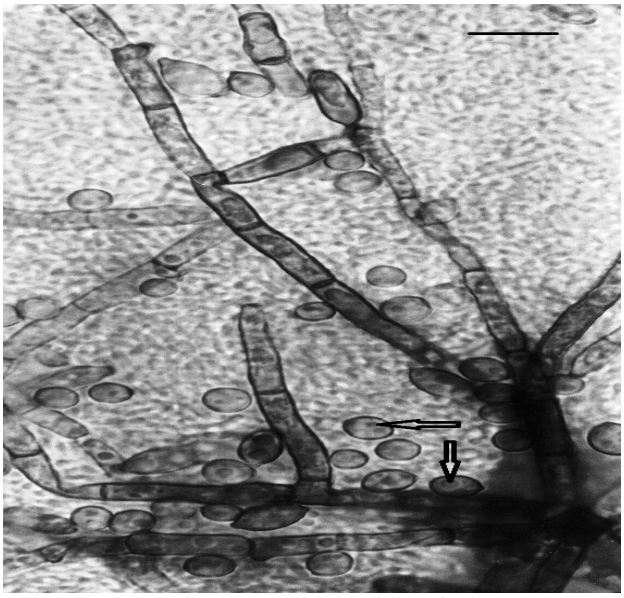


Fig. 10 – Conidiophores with conidia of Cladosporium cladosporioides (arrows). In the background you can see numerous cells of Bacillus subtilis. Scale of 10 micrometer

Being observed via the microscope such hyphae look empty and dead. Intrahyphae are not marked.

Hyphae and aerial mycelium cells changes are not observed.

Colonies of *Cladosporium cladosporioides* influenced by *Bacillus subtilis* culture are characteristic brown-green in color, however, there is no zoning, characteristic for colonies in control (Fig. 1 C). Sporulation of the fungus is not suppressed. Conidiophores are dark brown, solitary, erect, branched. Terminal branches are dichotomously branched, segmented, forming conidia at the ends. Conidia are collected in long chains, light, single-celled, ellipsoidal or lemon-shaped, (5,71 x 3,26) m in size.

Modifications in the morphology of conidiophores and conidia were not observed (Fig. 10).

In the substrate mycelium hyphae of *Fusarium sp*. (strain 46) under the influence of *Bacillus subtilis* culture little changes are observed. Like in the first two experiments, the cells of the hyphae becomes bloated (Fig. 11), however, hyphae do not die: inner contents and large central vacuole can be clearly seen (Fig. 12).

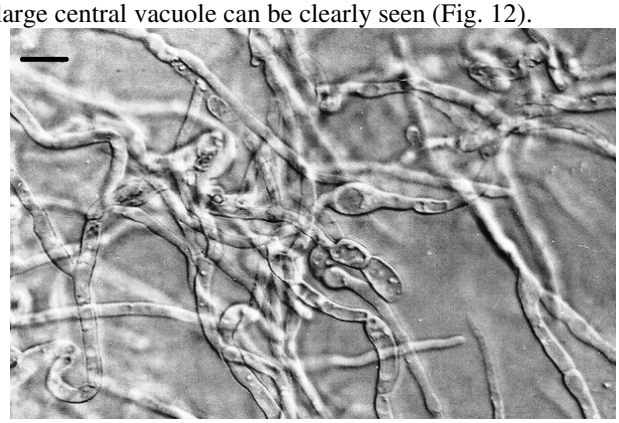


Fig. 11 – Hyphae of the Fusarium sp. substrate mycelium with swollen cells. Scale of 15 micrometer

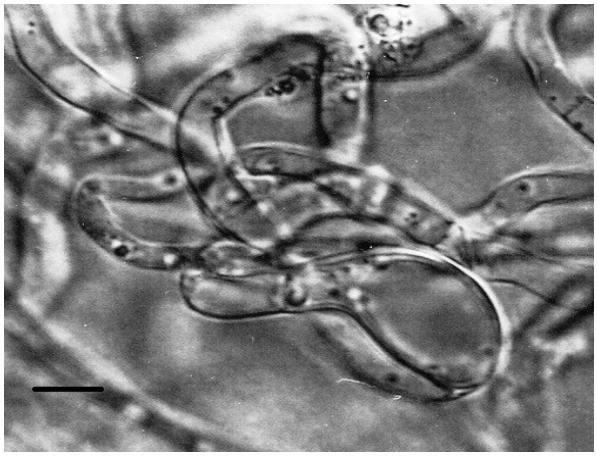


Fig. 12 – Swollen hyphal cell of Fusarium sp. substrate mycelium. Scale of 10 micrometer

On some hyphae of the aerial mycelium the rapid formation of lateral branches (presumable conidiophores) are noted conidiophores (Fig. 13). Characteristic of this isolate strands of hyphae formation and small structures (such as unpainted microsclerotia) appearance influenced by the culture of *Bacillus subtilis* are suppressed.

Under the influence of *Bacillus subtilis* culture colonies of *Fusarium sp*. become fluffy, white (in control - whitish colonies, fluffy, slightly zoned, fast-growing, covering almost entire dish, with pink center of the colony (Fig. 1 G). Macrosporulation is absent as in the original isolate. The formation of microconidia of not disturbed morphology is observed (Fig. 14).

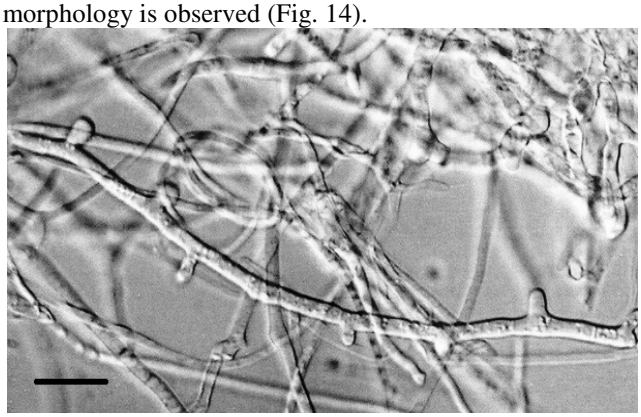


Fig. 13 – Fusarium sp. aerial mycelium hyphae with abundant branching. Scale of 20 micrometer

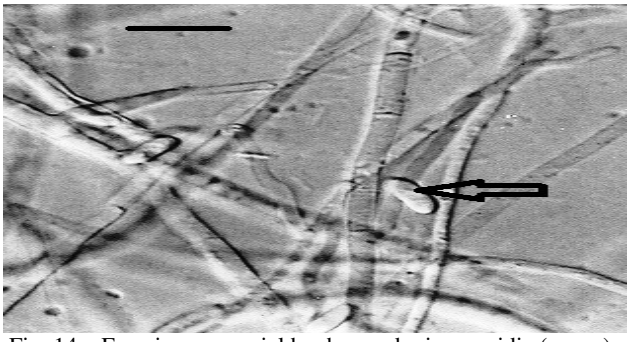


Fig. 14 – Fusarium sp. aerial hyphae and microconidia (arrow). Scale of 10 micrometer

## 4. CONCLUSION

Thus, we can conclude that under the influence of isolated from Ajania planoshaere *Bacillus subtilis* RKM B-0561 strain the growth and development of the studied pathogenic fungi are inhibited to varying degrees depending on their species. The resulting strain *Bacillus subtilis* can be used to develop a new kind of biological products to control plant pathogens.

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## REFERENCES

[1] P. Broadbent, K. F. Baker, N. Franks, and J. Holland. (1977). Effect of Bacillus species on increased growth of seedlings in steamed and in nontreated soil. *Phytopathology* [Online]. *67*. pp. 1027-1034.

[2] D. R. Fravel. (1988). Role of antibiosis in the biocontrol of plant diseases. Annu. Rev. *Phytopathol.* [Online]. *26*. pp. 75-91. Available: http://www.annualreviews.org/doi/abs/10.1146/annurev.py.26.090188.000451?journalCode=phyto

[3] M. H. Ryder, Z. Yan, T. E. Terrace, A. D. Rovira, W. Tang, and R. L. Correll. (1999). Use of strains of *Bacillus* isolated in China to suppress take-all and rhizoctonia root rot, and promote seedling growth of glass house grown wheat in Australian soils. *Soil Biol. Biochem.* [Online]. *31*. pp. 19-29. Available: http://www.sciencedirect.com/science/article/pii/S0038071798000959

[4] J. M. Whipps. (2001). Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.* [Online]. *52*. pp. 487-511. Available: http://jxb.oxfordjournals.org/content/52/suppl_1/487.abstract

[5] B. Chaurasia, A. Pandey, L. M. S. Palni, P. Trivedi, B. Kumar, and N. Colvin. (2005). Diffusible and volatile compounds produced by an antagonistic *Bacillus subtilis* strain cause structural deformations in pathogenic fungi *in vitro*. *Microbiological Research* [Online]. *160*. pp. 75-81. Available: http://www.sciencedirect.com/science/article/pii/S0944501304000849

[6] A. Pandey, and L. M. S. Palni. (1996). The rhizosphere effect of tea on soil microbes in a Himalayan monsoonal location. *Biol. Fertil. Soils* [Online]. *21*. pp. 131-137.

[7] A. Pandey, and L. M. S. Palni. (1997). *Bacillus species*: the dominant bacteria of the rhizosphere of established tea bushes. *Microbiol. Res.* [Online]. *152*. pp. 359-365.

[8] W. G. D. Fernando, R. Ramarathnam, A. S. Krishnamoorthy, and S. C. Savchuk. (2005). Identification and use of potential bacterial organic antifungal volatiles in biocontrol. *Soil Biology and Biochemistry* [Online]. *37*. pp. 955-964.

[9] J. Mercier, and D. C. Manker. (2005). Biocontrol of soil-borne diseases and plant growth enhancement in greenhouse soilless mix by the volatile-producing fungus *Muscodor albus*. *Crop Protection* [Online]. *24*. pp. 355-362.

[10] J. H. Bowers, and J. C. Locke. (2004). Effect of formulated plant extracts and oils on population density of *Phytophthora nicotianae* in soil and control of *Phytophthora* blight in the greenhouse. *Plant Diseases* [Online]. *88*. pp. 11-16.

[11] F. Lemessa, and W. Zeller. (2007). Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia. *Biological Control* [Online]. *42*. pp. 336-344.

[12] T. C. Paulitz, and R. R. Bélanger. (2001). Biological control in greenhouse systems. *Annual Reviews of Phytopathology* [Online]. *39*. pp. 103-133.

[13] S. J. Dong, L. P. Tredway, H. D. Shew, G. L. Wang, E. Sivamani, and R. D. Qu. (2007). Resistance of transgenic tall fescue to two major fungal diseases. *Plant Science* [Online]. *173*. pp. 501-509.

[14] A. Rodrigues, U. G. Mueller, H. D. Ishak, M. Jr. Bacci, and F. C. Pagnocca. (2011). Ecology of microfungal communities in gardens of fungus-growing ants (Hymenoptera: Formicidae): a year-long survey of three species of attine ants in central Texas. *FEMS Microbiol Ecol* [Online]. *78*. pp. 244-255.

[15] N. Tashiro, M. Noguchi, Y. Ide, and F. Kuchiki. (2013). Sooty spot caused by *Cladosporium cladosporioides* in postharvest Satsuma mandarin grown in heated greenhouses. *J Gen Plant Pathol.* [Online]. *79*. pp. 158-161. doi: 10.1007/s10327-013-0430-1

[16] P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and M. Grover, L. Nain, Sh. B. Singh, A. K. Saxena. (2010). Molecular and Biochemical Approaches for Characterization of Antifungal Trait of a Potent Biocontrol Agent *Bacillus subtilis* RP24. *Curr Microbiol* [Online]. *60*. pp. 99-106. doi: 10.1007/s00284-009-9508-6

[17] D. H. Bergey, J. G. Holt, N. R. Krieg, and P. H. A. Sneath. Bergey's Manual of Determinative Bacteriology (9th edn.). Lippincott Williams and Wilkins, 1994, ISBN 0-683-00603-7.

[18] K. Wilson. Preparation of genomic DNA from bacteria. *In:* Current Protocols in Molecular Biology. F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. (eds.). John Wiley & Sons, Inc., New York, 1997, pp. 2.4.1-2.4.2.

[19] W. G. Weisburg, S. M. Barns, D. A. Pelletier, and D. J. Lane. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* [Online]. *173*. pp. 697-703.

[20] S. F. Altschul, T. L.Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research* [Online]. *25*. pp. 3389-3402.

[21] H. L. Barnett and Barry B. Hunter Illustrared genera of imperfect fungi, Fourth Edition. New York, 1986, pp. 106, 130-132, ISBN 0-02-306395-5.

[22] J. H. C. Woudenberg, J. Z. Groenewald, M. Binder, and P. W. Crous. (2013). *Alternaria* redefined. *Studies in Mycology* [Online]. *75*. pp. 171-212. doi: 10.3114/sim0015

[23] K. Li, A. Moldagulova, E. Bekenova, A. Kazhibaev, M. Kairova, M. Urazova, S. Shaikhin, and K. Almagambetov. (2014). HAT-RAPD profiling of seven fungi isolated from central Kazakhstan soils. *South Asian Journal of Experimental Biology* [Online]. *4 (1)*. pp. 17-23.

[24] I. I. Novikova and Yu. D. Shenin. (2011). Isolation, Identification, and Antifungal Activity of a Gamair Complex formed by Bacillus subtilis M_22, a Producer of a Biopreparation for Plant Protection from Mycoses and Bacterioses. *Applied Biochemistry and Microbiology* [Online]. *47 (9)*. pp. 817-826.

[25] Liu Wei-wei, Mu Wei, Zhu Bing-yu, Du You-chen and Liu Feng. (2008). Antagonistic Activities of Volatiles from Four Strains of *Bacillus spp*. and *Paenibacillus spp*. *Agricultural Sciences in China* [Online]. *7(9)*. pp. 1104-1114.

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