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BACTERIA IN FUNGAL CULTURES ISOLATED FROM THE *SOLANACEAE* FAMILY PLANTS

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A B S T R A C T

A study of the presence and diversity of bacteria in cultures of fungi isolated from plants of the Solanaceae family (potato and tomato) was carried out using PCR with bacterial primers, followed by sequencing of the amplicons. A total of 83 strains were tested and bacteria were found in most of them. Bacteria of the following taxa were found in fungi: *Ceratobasidium* sp. *(Delftia* sp.), *Cladosporium cladosporioides (Paenibacillus* sp.), *Ilyonectria crassa (Enterobacter* sp.), *Fusarium avenaceum* (*Rahnella* sp.*, Stenotrophomonas* sp.), *F. equiseti* (*Pseudomonas* sp.*, Klebsiella* sp.*, Pseudomonas* sp.*, Pantoea* sp.*, Stenotrophomonas* sp.), *F. graminearum* (*Stenotrophomonas* sp.*), F. merismoides (Luteolibacter* sp.*), F. merkxianum (Stenotrophomonas* sp.), *F. oxysporum* (*Kosakonia* sp.*, Achromobacter* sp.*, Stenotrophomonas* sp.*, Pantoea* sp.*, Delftia* sp.*, Lelliottia* sp.*, Pseudomonas* sp.*), F. torulosum* (*Flavobacterium* sp.), *Orbilia oligospora* (*Lacrimispora* sp.), *Plectosphaerella cucumerina* (*Pantoea* sp.), *Pyrenochaeta* sp. (*Herbaspirillum* sp.)*, and Rhizoctonia solani* (*Achromobacter* sp.). No correlation was found between specific bacterial and fungal species. The impact of the identified bacteria on plants can vary, from involvement in pathogenesis to stimulating of growth, and needs further study. Bacteria associated with fungi can be used in the production of biological products with protective and growth-regulating effects. Combining such bacteria with non-pathogenic fungi will increase their survival; the resulting fungal-bacterial associations can be used to create growth-stimulating biological products with a long shelf life. The possible presence of dangerous bacteria in plant pathogenic fungi should be considered when developing plant protection measures.

Keywords: fungal-bacterial complexes, pathogenic bacteria, plant growth-promoting bacteria, microbial communities.

INTRODUCTION

To date, the relations between plants and bacteria and those between plants and fungi have been studied in nuance. However, the interaction between bacteria and fungi has not been studied in detail yet. Meanwhile, both endobiotic and ectobiotic bacteria are spread as widely among fungi as among animals and plants. A group of scientists from the USA, Brazil and Switzerland tested collection of fungal isolates from various taxonomic groups. Bacteria were found in most of the fungal isolates.

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Some strains had been preserved in the collection for many years, yet bacteria remained in them (Robinson e al., 2021). Bacteria can be attached to the surface of mycelium or live within it (Valdivia and Heitman, 2007). The role of the bacteria in the bacterial-fungal consortium is poorly understood and, apparently, can be different. Bacteria can be parasites, be neutral in relation to fungi or live in symbiosis with them (Bastías *et al.,* 2020). Of particular interest are fungal-bacterial-plants associations based on symbiotic relationships, since such a community of fungi and bacteria can enhance both positive and negative effects on the host plant.

A prime example of fungal-bacterial symbiosis is described in the article by Partida-Martinez and Hertweck (2005). *Burkholderia rhizoxinica,* endosymbiotic bacteria of *Rhizopus microspores*, are capable of producing rhizoxin,

which is toxic and disrupts normal functioning of rice plant cells. Such weakened plants are infected with *R. microspores,* and rice seedling blight develops. *R. microsporus* strains without *B. rhizoxinica* are not capable of infecting rice plants. Some bacteria capable of stimulating chlamydospore formation in mycelial fungi (Venkatesh *et al.,* 2022). Under laboratory conditions, *Ralstonia solanacearum* caused chlamydospore formation in fungi species from various taxa (Spraker *et al.,* 2016). Chlamydospores are thick-walled and are resistant to drying and temperature changes. Bacteria can survive harsh environmental conditions in chlamydospores together with fungi.

Bacteria can help arbuscular fungi form mycorrhizae. A study by Zhang *et al.* (2024) showed the beneficial effects of combining *Devosia* sp. with mycorrhizal fungi on plant growth and health.

In some cases, there is a clear correlation between fungi species and endosymbiotic bacteria. For instance, *Burkholderiaceae* endofungal bacteria are widespread in *Rhizopus* fungi. The authors consider symbiosis to be the result of evolution and that bacteria are vertically transmitted (Okrasińska *et al.,* 2021).

Apparently, endobiotic bacteria are widespread among various fungi species. However, their species diversity, impact on fungi life and the colonized plants need further examination. The presence of pathogenic bacteria closely related to fungi should be taken into account when planning plant protection measures (Platonov *et al.,* 2024). Potatoes and tomatoes are valuable food crops. Quite often, a whole complex of pathogenic organisms can be found in the lesion of these plants, and in order to preserve the health of these plants, it is necessary to consider the development of diseases as a multifactorial process, taking into account the relationships between fungi and bacteria. This aim of our research was a study of the presence and diversity of bacteria in the cultures of plant pathogenic and saprotrophic fungi isolated from plants of the Solanaceae family - potato and tomato.

MATERIALS AND METHODS

Samples of diseased fruits of tomato, potato tubers, leaves, and stems were collected from the commercial fields, storage facilities, and small private gardens in different regions (Table 1 and Figure 1). All samples were surface sterilized with sodium hypochlorite (2% solution) to remove possible contamination. Tubers and fruits were sliced across the damaged areas with a sterile blade. A slice of living infected tissue near the necrosis was transferred on plates with potato dextrose agar (PDA) amended with antibiotic (benzylpenicillin sodium salt, 100 mg/L). Leaves after sterilization put in wet chambers at 24 ± 1°C. For isolation, fungal spores or hyphae were taken from leaf surface using a preparation needle under a binocular microscope (MBS10, Russia), and transferred to culture media (PDA) amended with antibiotic.

Figure 1. Location of collecting sites.

Fungal strains were kept in the medium with an antibiotic (Penicillin G sodium salt, 1,000 units/ml). Visually, all studied strains had no signs of bacterial contamination. Species of the studied strains were identified based on cultural and morphological characteristics, and sequences of specific parts of the genome.

The mycelium of filamentous fungi for DNA extraction was grown in a liquid pea medium (Elansky *et al.,* 2022). After 5-7 days of incubation, the mycelium was separated from the liquid medium, dried on a filter paper, ground in a mortar with the addition of aluminum oxide, and the homogenized material was transferred into a 1.5 ml microtube. Subsequently, 800 µl of CTAB lysing buffer (100mM TRIS Ph 8.0; 1.4M NaCl, 20mM EDTA, CTAB solid 2% (w/v)) was added to the tube. The mixture was vortexed and then incubated for an hour in a water bath at +65°C. After incubation, 500 µL of chloroform was added, vortexed and centrifuged for 10 min at 13000 rpm. After centrifugation, the supernatant was taken and transferred to a clean microtube. At this stage, 400 µL of isopropanol + CH3COOK (1/10 vol, 5M, pH = 4.6) was added, gently mixed (by hand) and centrifuged for 10 min at the same speed. The supernatant was discarded, and the resulting pellet was washed with chilled 70% ethanol. It was centrifuged for 5 min at 13000 rpm, the alcohol was poured off, the procedure was repeated 3 times, the residual alcohol was removed with filter paper, and the precipitate was dried for 2–3 h. The pellet was then suspended in 50 µL of deionized water and stored at -20°C for future use.

For isolation of DNA from bacteria and yeasts, 3 mL of liquid culture, incubated for 18 h, was centrifuged at 5000 rpm for 5 min. The pellet was washed in 500 µL of TE buffer. After centrifugation, the pellet was suspended in 800 µL of CTAB buffer. Further, the procedure was carried out in the same way as in the isolation of DNA of filamentous fungi.

PCR was conducted using a Biometra T1 amplifier (Biometra, Germany). For each sample, 0.5 µl of 100 mM forward and reverse primers, 0.5 µl of dNTP (10 mM each), 0.5 µl of DNA polymerase (5 units/µl), 2.5 µl of 10x PCR buffer were taken. DNA fragments ITS1-5,8S-ITS2 (primers ITS4 and ITS5, (White *et al.,* 1990)) and *tef1* (EF1 and EF2 (O'Donnell *et al.,* 1998)) were amplified. To identify bacteria, PCR was conducted using bacterial primers for DNA fragments 16S rRNA (primers 27fc/519r-TTb) (Lane, 1991). The amplification program consisted of an initial denaturation step at 94°C for 1 minute, followed by 30 cycles of denaturation at 94°C for 30 seconds, primer annealing (at 52°C for ITS4/ITS5, 54°C for EF1/EF2, 51°C for 27fc/519r-TTb) for 30 seconds, and elongation at 72°C for 70 seconds. A final elongation step was performed at 72°C for 5 minutes. Each PCR experiment included both negative controls (Nucleic acid-free water) and positive controls (known DNA samples expected to yield an amplicon of a specific size). After the PCR reaction, the length and purity of the amplified DNA products were assessed using electrophoresis in a 1% agarose gel containing ethidium bromide (0.5 µg/mL). Once the electrophoresis was completed, a gel piece containing the single desired amplicon size was excised with a sterile scalpel and placed in a microtube. The extraction of DNA from the gel was performed according to the manufacturer's instructions specified in the CleanUp Standard gel kit (Evrogen Ltd, Russia). For DNA sequencing, the Sanger method was employed by the Evrogen Ltd company. The obtained DNA sequences were compared with existing sequences from the NCBI GenBank database. DNA sequence analysis was conducted using the MEGA 10 software for further investigation and identification of the isolated species.

Table 1. List of fungal strains taken for analysis for the presence of bacteria

Fungal name	Strain	Host plant, organ	Year of isolation	Place of collection (location on the Figure 1)	*
Acrostalagmus luteoalbus	21МПТ11/1	Potato tuber	2021	Russia, Moscow region (3)	
Alternaria alternata	19GaPT2	Potato tuber	2019	Gambia (11)	
A. alternata	19GaPT3	Potato tuber	2019	Gambia (11)	
A. alternata	20UgLaPT1-1	Potato tuber	2020	Uganda (12)	
A. alternata	21VNII2	Potato leaf	2021	Russia, Moscow region (3)	
Alternaria solani	20UgLaPT2_2	Potato tuber	2020	Uganda (12)	
Aureobasidium pullulans	21KKtepl1	Potato plant	2021	Russia, Kostroma region (2)	
Bjerkandera adusta	18CT1	Tomato fruit	2018	Russia, Moscow region (3)	
Ceratobasidium sp. AG-K	P1	Potato stem	2019	Russia, Astrakhan region (7)	+
Chaetomium globosum	18KVTF3-1	Tomato fruit	2018	Russia, Krasnodar region (6)	
Cladosporium cladosporioides	19GaPT5	Potato tuber	2019	Gambia (11)	+
C. cladosporioides	20UgLaTF10	Tomato fruit	2020	Uganda (12)	

Clonostachys solani	21MKpKK1	Potato tuber	2021	Russia, Moscow region (3)	
C. solani	21MKpKK3	Potato tuber	2021	Russia, Moscow region (3)	
Colletotrichum coccodes	C18U(G)PT4	Potato tuber	2018	Russia, Ussuri region (8)	
C. coccodes	C18TPS7	Potato stem	2018	Russia, Tatarstan (5)	
C. coccodes	Cc20UgKgPT1	Potato tuber	2020	Uganda (12)	
C. coccodes	Cc20UgLaPT1/1	Potato tuber	2020	Uganda (12)	
C. coccodes	Cc20UgKgPT 2	Potato tuber	2020	Uganda (12)	
Epicoccum nigrum	20UgLaTF 2/2	Tomato fruit	2020	Uganda (12)	
Fusarium avenaceum	23КамКК_Ф1	Potato tuber	2022	Russia, Kamchatka region (10)	+
F. avenaceum	23КамКК_18\1	Potato tuber	2022	Russia, Kamchatka region (10)	+
F. equiseti	18KTF22-1	Tomato fruit	2018	Russia, Krasnodar region (6)	
F. equiseti	20AKTL2\3	Tomato leaf	2019	Russia, Astrakhan region (7)	
F. equiseti	20UgTF1	Tomato fruit	2020	Uganda (12)	+
F. equiseti	20UgTF3	Tomato fruit	2020	Uganda (12)	+
F. equiseti	20UgLaTF1	Tomato fruit	2020	Uganda (12)	+
F. equiseti	20UgLaTF1-1	Tomato fruit	2020	Uganda (12)	
F. equiseti	20UgLaTF5-1	Tomato fruit	2020	Uganda (12)	+
F. equiseti	20 UgLaTF7	Tomato fruit	2020	Uganda (12)	+
F. equiseti	20 UgLaTF9	Tomato fruit	2020	Uganda (12)	+
F. equiseti	20UgLaPT1	Potato tuber	2020	Uganda (12)	
F. equiseti	20PT208	Potato tuber	2020	Uganda (12)	
F. equiseti	20PT211	Potato tuber	2020	Uganda (12)	
F. equiseti	20PT242	Potato tuber	2020	Uganda (12)	+
F. graminearum	20PT198	Potato tuber	2020	Uganda (12)	+
F. graminearum	20UgLaPT2_1	Potato tuber	2020	Uganda (12)	
F. merismoides	22Кам_3\2	Potato tuber	2022	Russia, Kamchatka (10)	+
F. merkxianum	F20AKPS3	Potato stem	2019	Russia, Astrakhan region (7)	+
F. oxysporum	20MKKK4	Potato tuber	2020	Russia, Moscow region (3)	
F. oxysporum	20UgLaTF4	Tomato fruit	2020	Uganda (12)	+
F. oxysporum	20PT195	Potato tuber	2020	Uganda (12)	+
F. oxysporum	20PT201	Potato tuber	2020	Uganda (12)	+
F. oxysporum	20PT203	Potato tuber	2020	Uganda (12)	+
F. oxysporum	20PT205	Potato tuber	2020	Uganda (12)	+
F. oxysporum	20PT206	Potato tuber	2020	Uganda (12)	+
F. oxysporum	20PT217	Potato tuber	2020	Uganda (12)	+
F. oxysporum	20PT241	Potato tuber	2020	Uganda (12)	+
F. oxysporum	20UgKgPT1/3	Potato tuber	2020	Uganda (12)	
F. oxysporum	20UgKacPT15	Potato tuber	2020	Uganda (12)	
F. oxysporum	21KPS4Vo	Potato tuber	2021	Russia, Kostroma region (2)	+
F. oxysporum	21B1.1	Potato tuber	2021	Russia, Moscow region (3)	+
F. oxysporum	21B3b	Potato tuber	2021	Russia, Moscow region (3)	+
F. oxysporum	21AEPS1	Potato stem	2021	Russia, Arkhangelsk region (1)	
F. sporotrichioides	14MPT17AB	Potato tuber	2017	Russia, Moscow region (3)	
Fusarium sp.	19EPTyaz1	Potato tuber	2019	Russia, Saratov region (5)	
F. sporotrichioides	14MPT17AB	Potato tuber	2017	Russia, Moscow region (3)	
Fusarium sambucinum	17Mikofag	Potato tuber	2017	Russia, Moscow region (3)	
F. solani	20AKKC5	Potato stem	2019	Russia, Astrakhan region (7)	
F.solani	20MKKK1.3	Potato tuber	2020	Russia, Moscow region (3)	+
F.solani	20MKKK3	Potato tuber	2020	Russia, Moscow region (3)	
F. solani	20PT204	Potato tuber	2020	Uganda (12)	
F. solani	20PT197	Potato tuber	2020	Uganda (12)	+
F. solani	21MKKK3	Potato tuber	2021	Russia, Moscow region (3)	
F. torulosum	22Kam2\2	Potato tuber	2022	Russia, Kamchatka (10)	+
Geotrichum candidum	21M11K	Potato tuber	2021	Russia, Moscow region (3)	

Helminthosporium solani	H17Ma(P)PT2	Potato tuber	2017	Russia, Magadan region (9)	
H. solani	H17Ma(S)PT7/1	Potato tuber	2017	Russia, Magadan region (9)	
H. solani	H18UKK4	Potato tuber	2018	Russia, Primorskiy region	
H. solani	H20UgKgPT3	Potato tuber	2020	Uganda (12)	
H. solani	H20UgKgPT8	Potato tuber	2020	Uganda (12)	
Ilyonectria crassa	17KSPT1	Potato tuber	2017	Russia, Moscow region (3)	+
Irpex lacteus	18KDTF6	Tomato fruit	2018	Russia, Krasnodar region	
Juxtiphoma eupyrena	17MaCKK1\4	Potato tuber	2017	Russia, Magadan region (9)	
J. eupyrena	17MaCKK1/8	Potato tuber	2017	Russia, Magadan region (9)	
J. eupyrena	17MaCKK4	Potato tuber	2017	Russia, Magadan region (9)	
J. eupyrena	17MaCKK6	Potato tuber	2017	Russia, Magadan region (9)	
Microdochium sp.	20PT213	Potato tuber	2020	Uganda (12)	
Orbilia oligospora	22Кам_3\1	Potato tuber	2022	Russia, Kamchatka region (10)	+
Plectosphaerella cucumerina	21MKKK2	Potato tuber	2021	Russia, Moscow region (3)	+
Pyrenochaeta sp	18KPTFan2/1	Tomato fruit	2018	Russia, Kaluga region (4)	+
Remotididymella destructiva	20UgMbPT4	Potato tuber	2020	Uganda (12)	
Rhizoctonia solani	19Chash_bf	Potato tuber	2019	Russia, Moscow region (3)	+

* – the "+" sign indicates fungal strains in which it was possible to determine the species or genus of associated bacteria

RESULTS

When PCR was carried out using bacterial primers for DNA extracted from fungi, the following problem appeared: PCR products of good quality, well detectable using electrophoresis, were obtained from only a half of studied isolates. However, even with such PCR products, it was possible to successfully determine the nucleic acid sequence not in all cases due to PCR product heterogeneity. Such heterogeneity occurs when the mycelium contains several species of bacteria at the same time, or if bacterial primers anneal a non-specific fragment of the fungal DNA. During DNA analysis of *Acrostalagmus luteoalbus, Alternaria alternata, A. solani, Cladosporium cladosporioides, Colletotrichum coccodes, H. solani, Geotrichum candidum*, *Irpex lacteus, Juxtiphoma eupyrena,* when PCR was conducted using bacterial primers, several amplicons of different sizes were produced. The PCR product based on bacterial primers was not synthesized for some fungal strains. The taxonomic affiliation of bacteria was identified for 30 fungi strains. The associated bacteria were of the following taxons: *Achromobacter* sp., *Acinetobacter* sp., *Delftia* sp., *Enterobacter* sp., *Flavobacterium* sp., *Herbaspirillum* sp., *Klebsiella* sp., *Kosakonia* spp., *Lacrimispora* sp., *Lelliottia* sp. *Luteolibacter* sp., *Paenibacillus* sp., *Pantoea* sp., *Pseudomonas* spp., *Rahnella* sp., and *Stenotrophomonas* sp. (Table 2 and Figure 2).

No correlation between bacterial and fungal species was revealed. *Pantoea* sp. was found both in *F. oxysporum* and *Plectosphaerella cucumerina*. *Stenotrophomonas* sp. was identified in the strains of three different *Fusarium* species. In *F. oxysporum* strains, eight different bacterial taxons were identified, while in *F. equiseti* there were six different bacterial species (Table 2). *Stenotrophomonas* and *Delftia* bacteria registered in various fungal strains were identical based on the studied sequences. *Pseudomonas* bacteria were significantly different. Figure 2 shows that they were divided into three clades and are apparently of different species. Strains of *Pantoea* and *Kosakonia* were also genetically different.

DISCUSSION

Plants live in symbiosis with a large variety of microbes. These microbes play an important role in improving nutrient availability for a plant, protecting it from pathogens and increasing stress tolerance. Complex relations are also established between different microorganisms forming the plant's microbiome. Bacteria associated with fungi can promote plant infection, utilization of complex substrates by fungi, and have effects on plants by producing specific chemical compounds which are identical to plant hormones. At the same time, fungi promote survival and spread of associated bacteria.

The bacteria identified in fungi strains include those which are close to plant growth-promoting bacteria. It was shown that *Achromobacter spanius* IP23 promotes plant growth by producing the "growth hormone", indoleacetic acid (Santos and Rigobelo, 2021).

Stenotrophomonas maltophilia SBP-9 improves wheat plant resistance to salt stress (Singh and Jha, 2017). *Klebsiella oxytoca* increases systemic resistance of potato and tobacco to PVY (Elsharkawy *et al.,* 2022). *Delftia* bacteria are well known as plant growth-promoting bacteria which also detoxify soil because they destroy some herbicides (Braña *et al.,* 2016). *Herbaspirillum* representatives also have growth-promoting properties (Monteiro *et al.,* 2012).

The trophic status of *Pseudomonas* bacteria differs. Some *Pseudomonas* bacteria associated with plants promote plant growth, suppressing pathogenic microorganisms, synthesizing plant hormones that stimulate growth and improving plant resistance to diseases. Other representatives of this genus lead to the disease (Preston, 2004). One of the *Pseudomonas* bacteria identified by us (20UgLaTF5-1) was close to *P. oryzihabitans* (MN565981, figure 2). This bacterial species is known to infect rice (Hou *et al.,* 2020). Another strain of *P. oryzihabitans* (MW187499), infects melon (Li *et al.,* 2021). Based on the studied fragment of the 16S gene sequence, one of the identified bacteria (B21.B1.1) is similar to *Lelliottia amnigena* (OK447935), which causes soft rot of potato tubers (Osei *et al.,* 2022). In some cases, the disease of potato tubers could be related to the presence of the pathogenic bacterium in the fungus.

Table 2. List of tested fungal strains and bacteria found in them.

Name of the fungus strain	Species name of the fungus	NCBI accession number	Name of the bacteria strain	Species name of the bacteria	NCBI accession number
P1	*Ceratobasidium sp.*	ITS: MW453064	B20AKKC1	*Delftia* sp.	OR381573
19GaPT5	*Cladosporium cladosporioides*	ITS: OR529207	B19GaPT5	*Paenibacillus* sp.	OR381570
17KSPT1	*Ilyonectria crassa*	ITS: MH818326	B17KSPT1	*Enterobacter* sp.	OR557619
23Kamf1	*Fusarium avenaceum*	ITS: OR591464	B23Kamf1	*Stenotrophomonas* sp	OR591467
23Kam18_1	*F. avenaceum*	ITS: OR591465	B23Kam18_1	*Rahnella* sp.	OR591490
20UgTF1	*F. equiseti*	ITS: OM421611	B20UgTF1	*Pseudomonas* sp.	OR470472
20UgTF3	*F. equiseti*	ITS: OM421613 TEF: OM362475	B20UgTF3	*Klebsiella* sp.	OR462712
20UgLaTF1	*F. equiseti*	ITS:OM421616 TEF OM362479:	B20UgLaTF1	*Pseudomonas* sp.	OR462688
20UgTF5-1	*F.equiseti*	ITS:OM421614 TEF: OM362476	20UgLaTF5-1	*Pseudomonas* sp.	OR462691
20UgLaTF7	*F. equiseti*	ITS:OM421617 TEF: OM362477	B20UgLaTF7	*Pantoea* sp.	OR462708
20UgLaTF9	*F. equiseti*	TEF: OM362480	B20UgLaTF9	*Stenotrophomonas* sp.	OR462710
20UgPT198	*F. graminearum*	ITS: OL364746	B20PT198	*Stenotrophomonas* sp.	OR460081
22KamPT3_2	*F. merismoides*	ITS: OR533484	B22Кам_3_2	*Luteolibacter* sp.	OR462725
F20AKPS3	*F. merkxianum*	TEF: ON409888	B20AKKC3	*Stenotrophomonas* sp.	OR459867
20UgLaTF4	*F. oxysporum*	ITS: OL372284	B20UgLaTF4	*Kosakonia* sp.	OL762470
20UgPT201	*F. oxysporum*	TEF: OM649882	B20PT201	*Achromobacter* sp.	OR460082
20UgPT203	*F. oxysporum*	ITS: OL372286	B20PT203	*Stenotrophomonas* sp.	OR460094
20UgPT205	*F. oxysporum*	TEF: OM649887	B20PT205	*Stenotrophomonas* sp.	OR460095
20UgPT206	*F. oxysporum*	TEF: OM649873	B20PT206	*Delftia* sp.	OR460096
20UgPT217	*F. oxysporum*	ITS: OL372287 TEF: OM649883	B20PT217	*Stenotrophomonas* sp.	OR460097
20UgPT241	*F. oxysporum*	ITS: OL372292	B20PT241	*Kosakonia* sp.	OR460099
20UgPT242	*F. oxysporum*	ITS: OM649874	B20PT242	*Pantoea* sp.	OR460188
21KPS4Vo	*F. oxysporum*	ITS: OR528743	B21KPS4Vo	*Delftia* sp.	OR462718
21MPTw1	*F. oxysporum*	ITS: OR528742	B21B1.1	*Lelliottia* sp.	OR462719
21MPTw3b	*F. oxysporum*	ITS: OR528741	B21B3b	*Pseudomonas* sp.	OR462716
22Kam2-2	*F. torulosum*	ITS: OR555824	B22Kam2-2	*Flavobacterium* sp..	OR557578
22KamPT3_1	*Orbilia oligospora*	ITS: OR531681	B22Кам_3\1	*Lacrimispora sp.*	OR462720
21MKKK2	*Plectosphaerella cucumerina*	ITS: OR529437	B21MKKK2	*Pantoea* sp.	OR462717
18KPTFan2/1	*Pyrenochaeta* sp	ITS: OR528638	B18KPTFan2_1	*Herbaspirillum* sp	OR381483
19Chash_bf	*Rhizoctonia solani*	ITS: OR531680	B19Chash_bf	*Achromobacter* sp.	OR459849

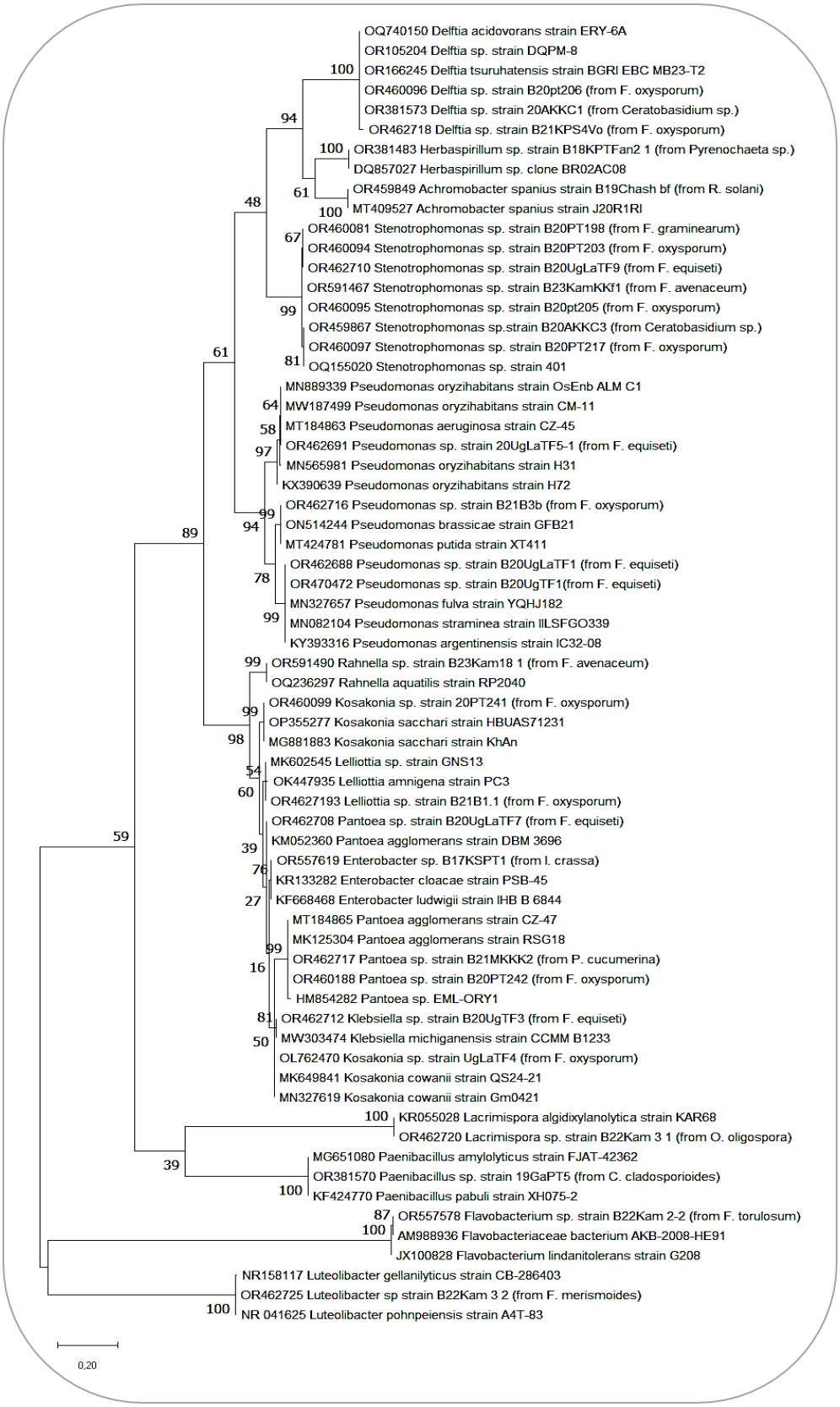

Figure 2. Phylogenetic tree inferred from maximum-likelihood analysis of the 16*S* gene region alignment. Bootstrap 1000 replicates. The figure also shows reference sequences.

Different strains belonging to the same bacterial species may have different degrees of pathogenicity or be non-pathogenic to the host plant. For example, *Kosakonia cowanii* strains are known to be pathogenic for soybean plants (Krawczyk and Borodynko-Filas, 2020). Another strain of *K. cowanii* was found to infect foxtail millet (*Setaria italica*) (Han *et al.,* 2023). At the same time, a non-pathogenic strain of *K. cowanii* was also described. Whole-genome sequencing of this strain revealed the absence of several virulence-related genes (Espinosa *et al.,* 2023). The *Pantoea agglomerans* strain (HM854282) has been described as a rice pathogen (Lee *et al.,* 2010), but another strain of this species, YS19, was non-pathogenic and had growth-promoting effects (Feng *et al.,* 2006). Thus, to study the properties of bacteria and their role in the fungus-bacterium-plant system, it is necessary to isolate axenic bacterial cultures.

The research showed that fungal-bacterial complexes are very strong. Bacteria were found even during the analysis of mycelium of the strains kept in the collection for several years which were periodically sterilized by antibiotics. According to pertinent literature, bacteria can play versatile roles. Associations of growth-promoting bacteria with non-pathogenic fungi are of interest for the development of biological drugs that stimulate plant growth. Many bacteria have been described that have a high potential for accelerating the growth and development of plants, however, due to the inability to form spores, such bacteria do not tolerate unfavorable environmental conditions. Combining such bacteria with non-pathogenic fungi will increase their survival; the resulting fungal-bacterial associations can be used to create growth-stimulating biological products with a long shelf life.

When protective measures are planned, it must be taken into account, that such measures are to cover not only fungi, but also fungal-bacterial complexes which may include plant pathogenic bacteria.

CONCLUSION

Many fungi are closely related to bacteria. Bacteria can spread and survive unfavorable conditions with the help of fungi.

Fungi can be in close association with phytopathogenic bacteria; such fungal-bacterial complexes can have a destructive effect on plants.

Based on fungi associated with growth-stimulating bacteria, highly effective biological products with a long shelf life, resistant to environmental influences, can be created.

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