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Adaptation of plant protection technology considering fungal-bacterial associations

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Abstract. Plant infections are often caused by complexes of microorganisms that may include both fungal and bacterial species. In some cases, a symbiotic relationship between fungi and bacteria is observed, which makes a significant contribution to the development of pathogenesis. The work analyzes the phytopathogenic potential of bacteria associated with fungi. Fungal cultures that did not have visible (including microscopy) symptoms of bacterial damage were selected for study. Using PCR with primers for bacterial mitochondrial genes followed by sequencing of amplicons in such fungal cultures, the presence of bacteria was established. Analysis of sequencing data showed that among the bacteria associated with fungi there were species related to known phytopathogenic bacteria that cause diseases of crops. The results obtained show the need to adjust plant protection measures. Most chemical fungicides are ineffective against bacteria. Protection schemes should include biological, biorational and chemical agents that can simultaneously control the development of fungi and bacteria. Crop rotations should be designed to avoid alternating crops susceptible to the same bacteria. Another important element of plant protection is the removal or destruction of plant residues.

Key words: phytopathogens, phytopathology, bacterial plant diseases, fungal-bacterial interactions, fungal-bacterial endosymbiosis

Conflict of interests. The authors declare that they have no conflict of interests.

Author Contributions: V.A. Platonov — data collection and processing, collection maintenance; S.N. Elansky — data analysis, writing the paper; E.M. Chudinova — conducting experiments, experiment design planning, writing the paper.

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Introduction

According to the UN, annual global losses of potential yield of all agricultural crops from pests and diseases amount to about 23 % of the gross harvest. Very often, the affected parts of plants contain a whole complex of pathogenic microorganisms. Sometimes it is difficult to determine which microorganism is the root cause of plant damage. In some cases, a symbiosis of fungi and bacteria is possible, which contributes to more effective damage to the plant. The most famous example of such interaction is described in the article by Partida-Martinez and Hertweck [1]. The bacteria *Burkholderia rhizoxinica*, endosymbionts of the fungus *Rhizopus microsporus*, are capable of secreting the toxin rhizoxin, which disrupts normal functioning of rice plant cells. Reduced immunity in rice plants allows the fungus *R. microsporus* to invade plant tissue. *R. microsporus* fungal strains lacking the endobiont bacteria *B. rhizoxinica* are unable to infect rice plants. Some bacteria can stimulate formation of chlamydospores, thick shell of which allows them to survive drying out and temperature fluctuations. In laboratory conditions, *R. solanacearum* caused chlamydia formation in 34 species of filamentous fungi from different taxa. Moreover, *R. solanacearum* was shown to penetrate the chlamydospore [2]. Apparently, bacteria can survive harsh environmental conditions in chlamydospores. Endobiont bacteria are widespread among members of fungal kingdom. A group of scientists from the USA, Brazil and Switzerland tested about 700 collection strains of fungi belonging to different taxonomic groups. Bacteria were found in most fungal cultures, including the cultures that were kept in the collection for several years [3]. Complex symbiotic and pathogenic relationships between plants, fungi and bacteria are currently poorly understood, but they must be considered when developing plant protection control measures.

The purpose of the study was to study bacteria associated with fungal hyphae isolated from plants of the Solanaceae family, as well as to assess phytopathological potential of bacterial component.

Materials and methods

In the research, we used fungal strains from the collection of pure cultures of the Agrobiotechnological department, Agrarian and Technological Institute, RUDN University. All studied strains had no visible symptoms of contamination with other microorganisms, including bacteria. The fungi were incubated on potato-glucose agar with the addition of penicillin (benzylpenicillin sodium salt, 1 million units/L). To isolate DNA, fungal mycelium was grown in liquid pea medium. DNA was isolated as described in [4], after which a PCR reaction was carried out using bacterial primers to the DNA region of 16S ribosomal RNA (27c/519r-TTb) [5]. PCR products were separated using agarose gel electrophoresis and the presence of the PCR product was visualized on a trans-illuminator. If one clear band was visible on phoresis, the PCR product was excised from the gel, purified and sequenced. The Cleanup Mini Kit (Evrogen, Russia) was used for DNA purification. To determine taxonomic affiliation of the analyzed bacteria, the obtained

sequence was compared with sequences deposited in Genbank NCBI database using Blast program. If necessary, phylogenetic constructions were used using Mega 10 program.

Results and discussion

Presence of bacteria was detected in 28 strains of fungi from the following species: *Ceratobasidium* sp., *Cladosporium cladosporioides*, *Ilyonectria crassa*, *Fusarium avenaceum*, *F. equiseti*, *F. graminearum*, *F. merismoides*, *F. merxianum*, *F. oxysporum*, *F. torulosum*, *Orbilia oligospora*, *Plectoshaerella cucumerina*, *Pyrenochaeta* sp., *Rhizoctonia solani* which were isolated from potato and tomato plants. These bacteria belonged to the following taxonomic units: *Achromobacter* sp., *Acinetobacter* sp., *Clostridium* sp., *Delftia* sp., *Flavobacterium* sp., *Herbaspirillum* sp., *Klebsiella* sp., *Kosakonia* sp., *Lelliottia* sp., *Luteolibacter* sp., *Pantoea* sp., *Pseudomonas* sp., *Rahnella* sp., *Serratia* sp., *Stenotrophomonas* sp.

According to the literature data, some of these bacterial genera can be pathogenic for plants. The bacterium *Lelliottia* sp. {OR462719} (hereinafter in curly brackets the numbers of sequences deposited in the Genbank NCBI database are given) claims to be a potato pathogen, the presence of which was discovered in *Fusarium oxysporum*, isolated from a potato tuber in the Moscow region. Strain PC3 *Lelliottia amnigena* {OK447935} was found on potato tubers (*Solanum tuberosum* L.) with macerated pulp and obvious signs of bacterial damage in the vicinity of Lanzhou (China) [6]. Another species of pathogenic *Lelliottia* was found on a plant from the bellflower family *Codonopsis pilosula* in China [7]. In a strain of *Fusarium oxysporum* isolated from a potato tuber grown in Uganda, we found bacteria belonging to *Kosakonia* genus {OL762470}. *Kosakonia cowanii* {MN327620}, similar in sequence, infects leaves of soybean (*Glycine max*) [8]. Strains of *K. cowanii* are known to cause wilting of plants from the Lamiaceae family *Pogostemon cablin* [9] and bacterial wilt in mogar (*Setaria italica*) {ON125560} [10]. Bacteria belonging to *Pantoea* genus, which were also identified in cultures of fungi of *Fusarium* genus from our collection {OR462708, OR460188}, can also exhibit pathogenicity. According to the literature data, *Pantoea agglomerans* {HM854282} can infect rice seedlings (*Oryza sativa*) [11], cause rotting of onions (*Allium cepa*) [12], and cause leaf spot in Chinese taro (*Alocasia cucullata*) [13]. Strain HXJ {HM016799} causes browning and premature fruit drop in walnut (*Juglans regia*) [14], strain PGHL10 {EF050809} causes leaf spot in corn (*Zea mays*) [15]. *P. agglomerans* pv. *gypsophylae* stimulated the appearance of galls in *Gypsophila paniculata*, *P. agglomerans* pv. *betae* infects beets (*Beta vulgaris*), and pathogenicity of these bacteria is due to the presence of a plasmid [16]. One of the bacteria we identified, belonging to the taxon *Pseudomonas* {OR462691}, is related to strains of *Pseudomonas oryzihabitans* that infect rice (*Oryza sativa*) [17], melon (*Cucumis melo*) {MW187499} [18] and walnut (*Juglans regia*) {OR195734} [19].

Thus, fungi isolated from plants of the Solanaceae family are associated with bacteria that can be pathogenic not only for plants of this family, but also for a wide range of other plants. Close association of fungi with bacteria and ability to jointly develop

pathogenesis should be considered when developing plant protection systems. Bacteria are more vulnerable, they tolerate drying out and temperature fluctuations worse than fungi; however, ability of bacteria to penetrate mycelium or spores of fungi makes bacterial infections more dangerous. Most chemical fungicides are ineffective against bacteria. To combat bacterial lesions, biological agents are used based on *Bacillus amyloliquefaciens*, *B. subtilis*, metabolites of *Streptomyces* spp. (macrolide tylosin complex, phytobacteriomycin, kasugamycin), colloidal silver and preparations containing iodine are also used¹. Some agents control development of fungi and bacteria simultaneously; they should be given preference in protecting plants from fungal and bacterial infections. Also, an effective way to combat diseases is the correct organization of crop rotation. However, since fungi can be carriers of bacteria pathogenic for various plants, planning crop rotation should be more accurate. Plant protection against fungal and bacterial diseases undoubtedly requires measures for timely removal or destruction of plant residues.

Conclusion

Many fungi are closely related to bacteria. Bacteria can spread and survive unfavorable conditions using fungi. Pathogenicity of fungi may be due to a bacterial component, and therefore fungicides may not have the desired protective effect.

To combat fungal-bacterial infections, crop rotations should be thoroughly designed, biological and chemical agents that control development of both fungi and bacteria should be included in the protection system, and plant residues should be removed or destroyed.

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Адаптация технологии защиты растений с учетом грибо-бактериальных ассоциаций

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Аннотация. Поражение растений часто вызывается комплексами микроорганизмов, которые могут включать как виды грибов, так и бактерий. В некоторых случаях наблюдаются симбиотические отношения грибов с бактериями, что вносит существенный вклад в развитие патогенеза. Проанализирован

фитопатогенный потенциал бактерий, находящихся в тесной ассоциации с грибами. Для изучения отбирали культуры грибов, не имевшие видимых (в т.ч. при микроскопировании) симптомов поражения бактериями. Методом ПЦР с праймерами на бактериальные митохондриальные гены с последующим секвенированием ампликонов в таких культурах грибов установили присутствие бактерий. Анализ данных секвенирования показал, что среди ассоциированных с грибами бактерий присутствуют виды, родственные известным фитопатогенным бактериям, вызывающим заболевания культурных растений. Полученные результаты показывают необходимость корректировки мероприятий по защите растений. Большинство химических фунгицидов неэффективны в отношении бактерий. В схемы защиты следует включать биологические, биорациональные и химические препараты, которые могут одновременно контролировать развитие грибов и бактерий. Севообороты следует проектировать таким образом, чтобы избежать чередования культур, восприимчивых к одним и тем же бактериям. Также важным элементом защиты растений является удаление или уничтожение растительных остатков.

Ключевые слова: фитопатогены, фитопатология, бактериальные болезни растений, грибо-бактериальные поражения, симбиоз грибов и бактерий

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