The Revealing of New Phytopathogenic Bacteria in Ukraine

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Abstract

After 15 or more years after accident at the Chernobyl nuclear power plant there was isolated a number of new for Ukraine phytopathogenic bacteria. All of them are characterized by a very high aggressiveness, which is preserved at the storage of bacteria in the laboratory. Among these bacteria are called: Erwinia amylovora, Curtobacterium flaccumfaciens and Curtobacterium sp. – previously missing in Ukraine, strongly aggressive Pantoea agglomerans – previously deemed to epiphyte, Pantoea agglomerans - not described in literature and a new form or, perhaps, a new species with some similarity of properties Clavibacter michiganensis subsp. michiganensis. In article there were presented some features of symptoms of bacterial diseases, caused by these agents.

Keywords

Phytopathogenic bacteria, The Chernobyl Accident, Fruit-trees, Ornamental Trees, Weeds, Soy-bean, Tomatoes

1. Introduction

Over the past few decades, the ecological situation in Ukraine has deteriorated significantly, due to the high technogenic loads, chemicalization in agriculture, the accident at the Chernobyl nuclear power plant, global warming on the continent, acid rains and other factors. All this leads to pollution of the environment and is reflected in a variety of living organisms, including microorganisms. The last evolve, migrate, adapt to the new environment.

Over the years of study of bacterial diseases of plants and their agents in Ukraine, as well as analysis of the literature, we noticed the appearance of new agents of bacterial diseases in the last years, on what we would briefly stop in this report.

2. Materials and methods

The objects of study were different types of plants, collected on the territory of Ukraine. Phytopathological analysis of the affected rhizomes, fruits, stems, leaves, buds, isolation of bacteria and study of their biological properties were performed, using standard techniques [2]. Pathogenic and virulent properties of isolates were determined by artificial inoculation of plants in vitro and in vivo. In all versions the bacterial suspension was used (at a concentration of 10⁹ cells/ml of sterile water), which was drifted on the surface of buds, leaves, of plants, grafts of plants, followed by needle injury. The repetition of experiments is five or seven times. The plants, infected by artificial method were under observation for 3 weeks in vitro, for 2 months in vivo. Results of artificial inoculation were recorded according to 5 gale scale. The bacteria were identified according to Bergey [19].

3. Results and discussion

1. Erwinia amylovora - an agent of fire blight of fruit is widely distributed in USA and Western Europe. On other continents, there are some reports on the detection of the causative agent, although not always these data were considered reliable. For years, the agent of bacterial blight of fruit was considered the only representative of bacteria A 2 List (The list of quarantine objects in Ukraine) [10]. It is a quarantine object to other countries, including Russia, Belorus [13]. For many years there was a view, that there is no fire blight in Ukraine. However, in 1997, for the first time in Ukraine was exposed agent of fire blight of pear [11]. The focuses of the disease were reported in the Transcarpathian and Chernivtsi regions [8,11]. This agent was revealed on the quince-trees in Odessa region [7]. Since 2005, we isolated bacteria E.amylovora from samples of fruit-trees and ornamental trees [15].

In the Kiev region we isolated bacteria E.amylovora from samples of apple-tree, pear-tree, rowan-tree, Cornelian-cherry-tree, various species of the genus Prunus, introduced from the Western European countries. In 2011 E. amylovora was isolated from patterns of two years of apple-trees, planted on an area 53 hectares in the Vinnitsa
region. Seedlings were imported from Hungary. In Ukraine investigators reveal new focuses of fire blight of fruit and agent _E. amylovora_ conquers new territories in this country. In this cage, we documented massive infection of different species of the genera _Malus_ and _Prunus_, although the latter, according to the authors, are not susceptible to this disease [8]. The manifestation of fire blight of fruit in Ukraine has some features: in Ukraine this disease manifest itself not only in the spring, but also in summer. At the summer development of the disease typical, twisted apicaluses of young shoots are absent and secretion of exudate is very rare. Even in the period May – June, researchers noted secretion of exudate not from all sorts of fruit-trees and only sometimes. We were isolating this agent during all vegetative period, beginning from April to October. However, in July and August bacteria are isolated in small numbers.

2. _Pantoea agglomerans_ is a typical representative of epiphytic microflora. Some researchers have attributed these bacteria to the weakly pathogenic bacteria, that can sometimes affect some of the plants under conditions, which are favourable for the development of microorganism [1,11]. However, in the literature there is evidence, that in 2004 opportunistic bacteria _P. agglomerans_ were the cause of mass disease of the collection and industrial crops of soy-bean of the Institute of Agriculture of UAAS [5]. According to data of the authors, the disease was on the verge of epidemics. Since 2002, over the years highly aggressive strains of _P. agglomerans_ were repeatedly isolated from affected patterns of _Elytrigia repens_ (L.) Nevski, _Arrhenatherum elatius_ (L.) J. et C. Presl, _Equisetum arvense_ L., growing in crops of wheat and soy-beans. In artificial infection isolated strains affected in different degrees a number of industrial crops, including wheat, kidney beans. [4,14,16]. The reason for this aggressive outbreak of _P. agglomerans_ in nature in relation to different plants remains unknown. Some strains have not lost their aggressiveness on the number of years, when stored on potato agar in the laboratory.

3. _Curtobacterium flaccumfaciens_ – it is the only species of phytopathogens of the genus _Curtobacterium_, 4 pathovars of which affect bean cultures and beets, tulips, poinsettia. Members of the genus _Curtobacterium_ previously have been identified as missing in Ukraine. However _C. flaccumfacien_ was exposed in 2009 as the causative agent of rusty-brown spot of soy bean in Ukraine [6]. Bacteria _Curtobacterium sp._ were isolated from the samples of _Equisetum arvense_ L. in the Kiev region. Since 2007 bacteria _Curtobacterium sp._ gradually become the main and the only bacterial pathogens of _Equisetum arvense_ L. in the surveyed region [16]. Probably, such conformity is associated with an increase in temperature indices during the growing season in those years. As is known, the optimum temperature for the development of plant pathogens, belonging to the genus _Curtobacterium_ – 31° C, maximum – 36°-40° C, the agent is killed at a temperature 60° C, it is resistant to the action of sunlight [1]. The authors do not exclude the possibility, that the isolated highly aggressive pathogens represent a single new pathovar of species _C. flaccumfaciens_.

4. _Micrococcus sp._ Phytopathogenic _Micrococcus sp._ were isolated from the samples of massively withering chestnuts in Kiev [17, 18]. Bacteria are aerobes, grow for 8-10 days after sowing of samples, in the laboratory on nutrient media quickly lose viability. The studied _Micrococcus sp._ is characterized by high aggressiveness with respect to plant-host. Within a few years, bacteria do not lose aggressiveness, while maintaining in vitro. In artificial infection they cause drying of buds and necrosis on leaves. Necrotic lesions are large, occupying 1.3-1.2 of leaf blade, often resembling a fire blight. Severely affected leaves wither and fall prematurely. The infected buds do not bloom into leaf at all or they are withering at the appearance of the first leaflets.

Phytopathogenicity is not peculiar to microorganisms of the genus _Micrococcus_. At present in the scientific literature as a pathogen was described only one type of _Micrococcus_ varians Cohn,, that causes stem rot of Musa textiles under unfavourable conditions of cultivation in Hondurus, Costa Rica, Panama [20]. Isolated by us bacteria _Micrococcus sp._ do not cause rot processes and are, apparently, a new phytopathogen, that causes necrosis of leaves of horse-chestnut, symptoms of which have not been described previously by other authors.

5. _Clavibacter michiganensis subsp. michiganensis_ is an agent of bacterial canker of tomato, one of the most common and harmful disease of tomatoes of open ground and greenhouse [3]. The disease is common in America, Europe, South Africa, Australia and the former Soviet Union. In the analysis of samples with symptoms of bacterial canker of tomato from greenhouses of the Kyiv region, along with typical representatives of bacteria _C. michiganensis subsp. michiganensis_, we have isolated bacteria, which grow in the form of a giant-grained translucent granular colonies – rough (R-) forms of colonies. When sowing bacteria by needle into the center of a Petri dish with potato agar after 20-24 hours colony occupied the entire surface of the agar, that is her diameter was equal to the diameter Petri dish. Because of this rapid growth we named these bacteria “creeping”. According to culture-biochemical properties and fatty acid composition of the cells, bacteria did not differ from the reference strains _C. michiganensis subsp. michiganensis_. However growth of isolated bacteria was not inhibited by antagonists (strains of bacteria of the genus Bacillus) to _C. michiganensis subsp. michiganensis_. In artificial infection isolated bacteria were more aggressive, the symptoms of infection were manifested within 7 days, the area of affection of stem vessels was extended to 15-20cm from the injection site, core of stem was affected too, infected plants wilted. The bacteria caused necrosis on tomato fruits. At the same time in the injection of tomatoes by reference strains _C. michiganensis subsp. michiganensis_ symptoms of wilting were manifested much slowly and weaker at 12-14 days after infection, the vessels of the stem were affected only on 5-7cm. The affection of the stem core is also not typical of the pathogen _C._
have been genetic changes in the nature of the existing species of bacteria. This article presents types of bacteria, isolated after the Chernobyl accident or any other factors (such as the influence of pesticides, used in greenhouses, or a new species of bacteria can be answered only genetic research.

4. Conclusion

Thus, we have isolated and identified a number of new agents of diseases for Ukraine. All of them are characterized by a very high aggressiveness, which lasts for a number of years of storage of bacteria in the laboratory. Biological properties of pathogens and features of the diseases, caused by these pathogens, have some features and differences from the earlier description of types of plant pathogens, that must be considered, when examining the various plants. Probably, among described agents we revealed new species of pathogens or we deal with the fact, that under the influence of the Chernobyl accident or any other factors (such as the movement of plasmids in a natural population) there have been genetic changes in the nature of the existing species of bacteria. This article presents types of bacteria, isolated after 13-15 and more years after the accident at the Chernobyl nuclear power plant. Due to the extremely high aggressiveness of these bacteria is important to investigate the reasons of their appearance, as well as their distribution in other regions, the ways of transmission of infection, the issues of systematic position of pathogens, the ways of reduction of their damage and more.

REFERENCES