BIOTECHNOLOGICAL CONTROL METHODS AGAINST PHYTOPATHOGENIC BACTERIA IN TOMATOES

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Abstract. Due to the lack of control over the spread of pathogens and effective pesticides with antibacterial activity bacterial diseases cause significant economic losses in vegetable production including tomatoes. Therefore, the search for ways of biocontrol of bacterial diseases pathogens in vegetable crops is an extremely urgent problem. The literature review presents a systematic analysis of modern measures of agrotechnical, chemical and biotechnological control of bacterial diseases in tomatoes, and increasing the resistance degree of these crops against phytopathogenic bacteria using traditional methods, cell selection and the involvement of genetic engineering approaches. It is shown that agrotechnical measures are preventive in nature, while using chemicals and antibiotics has side effects, in particular phytotoxicity and the appearance of resistant strains of pathogens. Effective and economically viable is the use of biotechnological preparations and the cultivation of the varieties resistant to pathogens of bacterial diseases in vegetable crops. Selection of varieties resistant to phytopathogenic bacteria is based on the use of wild species as resistance sources. The creation of genetically modified plants containing avr-genes, resistance genes against bacterial phytotoxins, PR-proteins and AMP is promising.
Keywords: *bacterial diseases, biopreparations, antagonists, cells selection, GM-resistant plants*

Introduction

One of the reasons for the limited production of vegetable products is the significant economic losses caused by bacterial diseases (Khaliluev and Shpakovski, 2013). In vegetable crops bacterial etiology describes 40 widespread pathogens (Hvozdiak et al., 2011). An intensive growth in harmfulness has been recently observed in the case of phytopathogenic bacteria, which is the result of: 1) the emergence of new bacteria strains affecting a wide range of vegetable crops; 2) global climate change, contributing to the expansion of areas of bacteria distribution; 3) lack of reliable control over the infection sources and circulation of pathogens; 4) introduction of new varieties and technologies of plant cultivation; 5) excessive fungicide application (Punyna, 2009).

The basis for the developing control methods for bacterial pathogens is timely and accurate diagnosis. After all, bacterial diseases often have external manifestations similar to the symptoms of diseases caused by micromycetes and mycoplasmas (Hvozdiak et al., 2011). Imperfect laboratory diagnosis leads to improper or even harmful use of the means meant to control pathogens.

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Modern bases of protection of vegetable crops from bacterial diseases are based on complex use of methods of control of pathogens (Khaliluev and Shpakovski, 2013). For their effective use, it is necessary to reliably determine the direction of existing methods with respect to the nature and pathways of pathogens and sources of infection. The review presents agrotechnical and chemical methods, the use of biotechnological preparations, antibiotics, the cultivation of resistant and genetically modified plant varieties, wish are part of a complex system for control of tomato bacterial diseases agents.

Agrotechnical measures

Compliance with optimal temperature regimes (avoiding sudden changes between night and day temperatures), air and soil humidity, planting time, timely application of fertilizers and irrigation with water the temperature of which is not higher than 25 °C. In greenhouses, treating the tomato seeds with disinfectants, removal of crop residues and disinfection of equipment are recommended. In the field, as a result of the intensive development of bacterial diseases, crop rotation is used with the return of the culture not earlier than in the season, the removal of weeds and plant residues, minimization of mechanical damage to the culture, the destruction of infected plants or pruning of infected leaves. However, the existing measures are only preventive in nature (Tkalenko, 2012; Huliaieva et al., 2018).

Chemical measures

In production conditions fungicides are used against pathogens of bacterial diseases, as there are no special preparations with antibacterial activity among those registered in Ukraine (Kolomiiets et al., 2017a). In our research we have been investigated antibacterial action of about 50 pesticides with such active ingredients as dimethomorph, mancozeb metalaxyl, azoxystrobin, fenamidone, propamocarb hydrochloride, copper sulfate and oxychloride, mandipropamid, difenoconazole, aluminium phosphide, phosphorous acid, pyraclostrobin and metiram included in the List of pesticides and agrochemicals and allowed for use in Ukraine on tomato crops. It can be argued that the vast majority of pesticides used, does not suppress the development of pathogens of bacterial diseases in tomato plants. Slight antibacterial activity against strains of the pathogens of bacterial cancer, bacterial speak, and black bacterial spot of tomatoes has only aluminium phosphide and phosphorus acid, as well as mancozeb and mancozeb in combination with metalaxyl (Kolomiiets et al., 2017a). From this point of view, it is important to search, test and develop the methods for the use of special substances for the control of phytopathogenic bacteria (Dankevych et al., 2018).

Now, to control bacterial diseases of tomatoes, preference is given to preparations, in particular, sulfate, oxychloride and copper hydroxide (Khadija, 2002). Copper ions denature proteins and thereby destroy enzymes, which are crucial for the bacterial cells functioning (Mohsin et al., 2016). Copper destroys the cells of pathogens on the surface of plant leaves, but once they penetrate the host tissue, they are no longer sensitive to copper processing. Thus, copper sprays act as protective fungicidal / bactericidal methods in the early stages of infection, but are not characterized by prolonged and stable activity (Behlau et al., 2008).

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Known that the use of copper hydroxide and mancozeb inhibits the development of bacterial cancer, which confirms a synergistic effect, as a separate application of mancozeb does not reduce the population and spread of *Clavibacter michiganensis* subsp. *michiganensis* (Hausbeck et al., 2000).

In our research we have found insignificant antibacterial activity of chemical defenses on the basis of copper hydroxide (770 g/kg) and mancozeb (800 g/kg) relative to gram-positive *C. michiganensis* subsp. *michiganensis* (Kolomiiets et al., 2017a).

The use of copper sulfate and 8-hydroxyquinoline resulted in a significant reduction in the symptoms of bacterial cancer of tomatoes was established (Leon et al., 2008). At the same time, a synergistic effect was observed of 8-hydroxyquinoline/ copper sulfate against *C. michiganensis* subsp. *michiganensis*. The combination of available substances in the conditions of reducing the concentration by half provided a more significant and reliable reduction of bacterial symptoms than the use of individual substances (Leon et al., 2008).

In our research we have established a slight antibacterial activity of copper sulfate (345 g/l) relative to *C. michiganensis* subsp. *michiganensis* with zones of growth inhibition of 20 – 50 mm. Relative to tomato bacterial mottle pathogen *P. syringae* pv. *tomato*, antibacterial activity was observed within the growth inhibition zones from 14 to 54 mm, and for the black bacterial spotting pathogen *X. vesicatoria* it did not exceed 18 mm (Kolomiiets et al., 2017a).

The use of bactericides based on copper in combination with fungicides, ethylene-bis-dithionate or MANCOZEB caused an increase in the level of control of even copper-tolerant populations of pathogens. The inductor of plant resistance acibenzolar-S-methyl (CGA-245704 or Actigard) ensured the formation of low indicators of control of the distribution of tomato black bacterial spotting and bacterial mottle (Itako et al., 2014).

It was found that hexanoic acid (Hx) reduces the development of symptoms caused by *P. syringae* pv. *tomato* DC3000 by 50% in treated plants when compared to untreated ones, which indicates the induction of Hx resistance against this pathogen (Scalschi et al., 2014). The effect of Hx is based on inactivation of bacterial virulence genes and slowing their expression or regression (Scalschi et al., 2014).

In the field (Itako et al., 2014) it was evaluated the effectiveness of acibenzolar-S-methyl, fluosines, pyraclostrobine, copper oxychloride, MANCOZEB/ copper oxychloride and pyraclostrobine/metiram for the control of tomato black bacterial spot. In 40 days after transplantation, the plants were inoculated with *X. perforans* (10^7 CFU/ml) in order to assess the degree of development of the disease. Promising results were obtained only under treatment with fluosine, pyraclostrobine, pyraclostrobine/metiram, copper oxychloride and mancozeb/copper oxychloride. The activity of enzymes of polyphenol oxidase and peroxidase was higher than in terms of application of acibenzolar-S-methyl, pyraclostrobine and pyraclostrobine/metiram on tomato leaves, which confirms their participation in the mechanisms of induction of resistance to the pathogen *X. perforans*.

In our research, in a series of studies, the preparation with the active substance pyraclostrobine (50 g/kg) metiram (550 g/kg) was somewhat active against gram-positive *C. michiganensis* subsp. *michiganensis* and did not show antibacterial activity against gram-negative *P. syringae* pv. *tomato* (Kolomiiets et al., 2017a). In our opinion, this is due to the structural features of the cell wall of gram-positive and gram-negative bacteria. This drug caused the maximum increase in the activity of the enzyme

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peroxidase in the leaves of plants for 12 h, which was 111.5–112.5 $u.mg^{-1} \cdot s^{-1}$ (Kolomiiets et al., 2017a).

However, in published literature chemical means of bacterial pathogen suppression is only partially effective, and under favorable conditions for the development of the disease (high temperature, precipitation) are generally ineffective. Intensive treatment with copper for commercial tomato cultivation for many years caused the appearance of resistant strains of phytopathogenic bacteria (Patyka et al., 2016), accumulation of copper in soils and water with subsequent toxic effects on plants, soil beneficial microflora and invertebrate organisms. As a result, there is an urgent need to develop fundamentally new alternative measures to protect vegetable crops from bacterial diseases.

Biotechnological preparations

The basis of the creation of biotechnological preparations of different functional orientation for biocontrol of phytopathogenic bacteria is the elective ability of microorganisms to exhibit antagonistic activity against phytopathogens and stimulate plant defense mechanisms. The prospects of creation of biological means of protection of vegetable crops on the basis of bacteria of the genus *Bacillus* are shown. It is believed that the high level of antagonism of bacilli against phytopathogenic bacteria is associated with the synthesis of a wide range of exometabolites (Bais et al., 2004; Stein et al., 2004; Butcher et al., 2007; Nagorska et al., 2007; Ongena et al., 2007; Ongena and Jacques, 2008; Roi et al., 2012). Anyway, the literature does not provide enough information about the exact mechanisms of action *B. subtilis* as an agent of biocontrol of pathogens on vegetable crops.

The antibacterial activity of surfactin (lipopeptide antimicrobial agent) was determinedby testing the mutant strain *B. subtilis* M1 with deletion in the surfactants gene, which was ineffective as a biocontrol agent of *P. syringae pv. tomato* DC3000 (Bais et al., 2004). Wild strain *B. subtilis* 6051 in terms of colonization of the roots formed stable and extensive biofilm and secreted surfactin, inhibiting the growth of the pathogen of tomato bacterial mottle, which confirms its bactericidal activity.

The treatment with the suspension *B. subtilis* (Quadra 136 and 137) and *Rhodosporidium diobovatum* (S33) prevents the development of bacterial cancer in greenhouse conditions, which is caused by *C. michiganensis* subsp. *michiganensis* was reported (Utkhede and Koch, 2004). Similarly, in terms of treatment of seeds with strains *B. subtilis*GBO3, *B. amyloliquefaciens* IN937a and *Brevibacillus brevis* IPC11 maximum protection of tomatoes against bacterial cancer was recorded.

In our research experiments, biological preparations Phytohelp, Phytocid and Extrasol on the basis of *B. subtilis* had different antibacterial activity to pathogens. Thus, Phytohelp and Phytocid showed high antibacterial activity against *C. michiganensis* subsp. *michiganensis* and *X. vesicatoria*, and the diameter of the zone of absence of growth ranged from 70 to 80 mm. For strains *C. michiganensis* subsp. *michiganensis* and *X. vesicatoria* in terms of action of the Extrasol preparation, it did not exceed 40 mm. Active to the causative agent of tomato bacterial mottle *P. syringae* pv. *tomato* was Extrasol with a diameter of the zone of growth absence of 20–26 mm. Other preparations did not affect this pathogen (Kolomiiets et al., 2017a).

It was described the antagonistic activity of endophytic bacteria *B. pumilus* and *B. amyloliquefacies* against *P. syringae* pv. *tomato* NS4, transformed by GFP-gene (Green

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Fluorescent Protein), and a wild-type NW strain. After using endophytic bacteria was monitored population decrease of *P. syringae* pv. *tomato* NW and NS4 on phyloplan, and number of bacterial spots on the leaves of tomatoes compared with standard chemical protection of the copper oxychloride. In terms of fluorescence microscopy it was monitored small number of labeled GFP cells *P. syringae* pv. *tomato* NS4, which colonized rich in the carbon organic compounds areas of phytoplan. Untreated with antagonists plant leaves contained a significant amount of labeled GFP cells *P. syringae* pv. *tomato* NS4 (Filho et al., 2013).

It was shown the efficiency of epiphytic bacteria *Paenibacillus macerans* and *B. pumilus* for biocontrol of *X. vesicatoria* (Lanna et al., 2010), that reduce by 70% the number of phytopathogenic bacteria cells in phytoplan. The test for antagonistic activity confirmed that epiphytic bacteria effectively inhibit the growth of phytopathogens.

According to data (Fousia et al., 2015), treatment of seeds with *B. subtilis* QST 713 significantly reduces the development of bacterial diseases and provides an increase in plant height when compared to the control. In addition, quantitative PCR-analysis of expression *PR1a*, *PR1b,* and *Pin2* (encoding enzymes for the biosynthesis of salicylic and jasmonic acids) confirmed the role of *Pin2* in protective activity of *B. subtilis* QST 713, as an ecspression of *Pin2* was significantly higher in the treated with *B. subtilis* QST 713 plants, infected by *P. syringae* pv. *tomato* when compared to the control. An early increase was determined in the activity of antioxidant enzymes of superoxide dismutase, catalase, peroxidase and polyphenol oxidase, and a decrease in the content of malonic aldehyde in terms of inoculation of *B. subtilis* QST 713, which plays a key role in reducing oxidative stress and induces systemic resistance of tomato plants against black bacterial spotting (Chandrasekaran and Chun, 2016).

Effective against pathogens of bacterial diseases were biopreparations based on bacteria of the genus *Streptomyces,* which are characterized by selectivity of action and high activity to phytopathogens in low concentrations, which allows avoiding their excessive accumulation in the fruits of vegetable crops. When compared to chemical products, they penetrate more intensively and are metabolized in plant tissues through the leaf surface, stems and roots, are less toxic, decompose quickly, do not pollute the environment and dominate most fungicides in terms of effectiveness (Ferraz et al., 2015).

To prevent loss of tomato crops in greenhouses, caused by the pathogen of bacterial wilt *Ralstonia solanacearum*, it was proposed the root treatment of plants with bacterial isolates *B. thuringiensis* CR-371 and actinomyces *S. avermectinius* NBRC14893 (Elsharkawy et al., 2015).

The possibility of using antagonists *S. setonii* UFV618 and *B. cereus* UFV592 to reduce the symptoms of black bacterial spotting and induce the synthesis of protective enzymes in the leaves of tomato plants that are infected by *Xanthomonas* has been established. The final degree of development of the disease decreased by 29.44 and 59.26% in treatments with *B. cereus* UFV592 and *S. setonii* UFV618. The activity of antagonists can be explained by the activation of protective peroxidase enzymes, polyphenol oxidase, β-1,3-glucanase, chitinase, phenylalanine ammonia-lyase and lipoxygenase involved in the formation of systematic resistance of plants against bacterial diseases (Ferraz et al., 2015).

The antibacterial activity of biopreparations based on *Streptomyces:* Avercom, Avercom nova, Violar, and Phytovit, synthesizing antibiotic substances active to a wide range of microorganisms and fungi have proved (Elsharkawy et al., 2015). Areas of no

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growth of strains *C. michiganensis* subsp. *michiganensis* in terms of action of piopreparations Avercom, Avercom nova, Violar i Phytovit were 16–50 mm. Biopreparations Phytovit and Violar ware inert the pathogens of tomato bacterial mottle *P. syringae* pv. *tomato* and black bacterial spot *X. vesicatoria* (Biliavska rt al., 2015).

Thus, in vegetable growing, promising is the use of biopreparations, which are based on living cultures and metabolic products of microorganisms. It was confirmed antagonistic activity of bacteria of genera *Bacillus* and *Streptomyces* to phytopathogenic bacteria, and biopreparations based on them are recommended for biocontrol of bacterial pathogens.

Antibiotics as means of protection of vegetable crops against bacterial diseases pathogens

Protection against bacterial diseases includes the treatment of vegetable crops with antibiotics, which have advantages in the fight against phytopathogenic microorganisms in comparison to chemicals. They easily penetrate into the tissues and organs of plants through the roots, stems, leaf surface and are metabolized in seeds, so their action is less dependent on climatic conditions; they have antibacterial effect, are relatively slow inactivated, and are non-toxic to the plant body (Kolomiiets et al., 2016). Especially quickly penetrate into plant tissue antibiotics of neutral and acidic nature (chloramphenicol, penicillin), slower – amphoteric (chlortetracycline, oxytetracycline) and antibiotics-the basics (neomycin, streptomycin) (McManus et al., 2002).

Antibiotics for plants are made in the form of powders that contain from 17 to 20% of the active ingredient, and are dissolved or suspended in water to a concentration of 50 to 300 parts per million, and then are applied as an aerosol to the plant organs susceptible to pathogens. They are relatively expensive, so they are primarily used in vegetable and fruit crops (McManus et al., 2002).

The most promising for vegetable production are streptomycin preparations, which suppress the proliferation of bacteria by binding to ribosomes and inhibiting protein synthesis at the stage of initiation of translation (Schluenzen et al., 2006; Schuwirth et al., 2006). The US environmental protection Agency assigned the lowest toxicity category and lack of carcinogenic and mutagenic activity to streptomycin and oxytetracycline. In New Zealand it was registered streptomycin-containing preparation Keystrepto™ for the control of *P. syringae* pv. *tomato, X. vesicatoria, C. michiganensis* subsp. *Michiganensis,* and *P. syringae* pv. *syringae* on tomatoes (Vanneste, 2011).

It was shown that neomycin from the liquid culture of the fungus *S. fradiae* HTP has antibacterial activity *in vitro* and *in vivo* against phytopathogenic bacteria *R. solanacearum, E. carotovora* and *X. vesicatoria*. In terms of concentration 200 mg^{-1} of neomycin the reduction in the degree of development of the disease is ranged from 69.07 to 80.51%, which is more effective than in terms of treating with streptomycin – from 50.00 to 72.56% (Tao et al., 2011).

In Florida (USA) it was estimated the influence of kasugamicine (commercial preparation Kasumin® 2L) on the pathogen of tomato bacterial mottle. During its application in the greenhouse there was a decrease in the degree of development of bacterial mottle by 37.5% when compared to the control (Vallad and Pernezny, 2010; EPA, 2005).

However, the use of antibiotics can cause the proliferation of antibiotic-resistant bacteria and the spread of antibiotic resistance genes in the environment or even in

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humans (McManus et al., 2002). So, the appearance of streptomycin-resistant (Sm^R) pathogens makes it difficult to control bacterial diseases of vegetable crops. For example, in the USA streptomycin is allowed to be used on tomato and pepper plants for the control of *X. campestris* pv. *vesicatoria*, which is rarely used for this purpose, as resistant strains were first discovered in Florida in the early 1960s, which are now widespread. Sm^R include the other phytopathogenic bacteria such as *E. carotovora*, *P. chichorii*, *P. lachrymans*, *P. syringae* pv. *papulans*, *P. syringae* pv. *Syringee*, and *X. dieffenbachiae*. The use of kasugamicine is also contradictory, which, together with streptomycin have similar biological mechanisms of action.

It becomes evident that antibiotics are ineffective in protecting plants against bacterial diseases through their instability, blocking metabolic pathways, phytotoxic side effects, entering the human and animal food chain, high cost and development of resistant bacterial populations. Promising is the use of non-preparative forms of antibiotics, and biotechnological preparations, which are based on strains-producers.

Cultivation of resistant varieties of vegetable crops is one of the promising systems of biocontrol of phytopathogenic bacteria. The problem of complex resistance of genotypes against the most dangerous diseases has not been solved yet (Khaliluev and Shpakovski, 2013). The reasons for this are the genetic complexity of the trait, genome instability and microevolutionary changes in the host-pathogen system, as well as the emergence of highly resistant biotypes of pathogens against the background of the use of increased amounts of pesticides (Khaliluev and Shpakovski, 2013), the gradual increase in the duration of the average temperatures of the growing season, the proportion of monoculture and genetic homogeneity of the varieties that are grown. To provide breeding programs, it is necessary to search for new sources of resistance against bacterial diseases, which will allow optimizing, accelerating and increasing the effectiveness of the breeding process, creating the new varieties and hybrids with high resistance against pathogens (Khaliluev and Shpakovski, 2013).

There is evidence that microscopy of the stems of resistant tomato plants affected by *R. solanacearum*, showed restrictions on the spread of bacteria with thickening of the cell membrane and synthesis of suberin. The available samples are recommended to be used in the program for the selection of tomatoes against the bacterial wilting pathogen (Kim et al., 2016). In our research, the tomato plants of the resistant variety Chaika under the action of virulent strain *P. syringae* pv. *tomato* IZ-28 the cell walls were seeped with suberin and filled with lignin components, which is typical for the reactions of induced immunity. Lignin was intensively deposited on tangent and frontal anticlinal walls according to the potential directions of translocation of phytopathogenic bacteria, which created cell barriers to their spread (Kolomiiets et al., 2017b).

The localization and distribution of *R. solanacearum* in plants of 11 resistant tomato varieties from different genetic sources and susceptible variety Ponderosa was studied (Nakaho et al., 2004). The spread of bacteria in the stems of resistant tomato plants was suppressed by blocking the transition of pathogens from protoxylem or primary xylem to other xylem tissues. It was most noticeable on the Hawaii 7996 breeding line, which may be an alternative genetic source for tomato plant reproduction, resistant to bacterial wilting.

In Bulgaria they were obtained stable against two races T1 and T3 *X. vesicatoria* tomato lines for growing in the field. Lines created by hybridization between wild species *Lycopersicon L. pimpinellifolium* PI 126925, *L. chilense* LA 460, *L. peruvianum* var *humifusum* PI 127829, and *L. hirsutum* f. *glabratum*. PI 134418, were used as

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sources of sustainability. They were tested more than a hundred lines of tomatoes, from which they were selected promising numbers 36, 44, 44/1, 163/1, 165/2, 167, 167/4, 267 and 270, which showed high resistance against the race T3 *X. vesicatoria*. Stable plants from the group I were obtained by hybridization with *L. pimpinellifolium* PI 126925. A significant number of stable lines were selected in the group II with the involvement of *L. chilense* LA 460 and *L. peruvianum* var *humifusum* PI 127829. Group III lines that occurred with *L. hirsutum* f. *glabratum* PI 134418, were less resistant to race T1. At the same time, their natural resistance against the T3 race has not changed. The available nine lines were marked by valuable morphological and agronomic traits, which were selected for the reproduction of resistant varieties of tomatoes and as a starting material in cross-breeding programs (Ivanova et al., 2006).

In Uruguay, where tomatoes are affected by the race T2 *X. vesicatoria*, were identified varieties Hawaii 7981, Loica and Ohio 8245, which can be used as new natural sources of resistance to the pathogen *X. vesicatoria* of the T2 race (Berrueta et al., 2016).

Now in the world market there are no varieties of tomatoes that would be resistant against *C. michiganensis* subsp. *michiganensis*. It was provided the screening of wild tomato species for resistance against *C. michiganensis* subsp. *michiganensis* (Sen et al., 2013). High tolerance was revealed in *S. arcanum* LA 2157, *S. peruvianum* PI 127829 i *S. arcanum* LA385 and average – in *S. habrochaites* LA 407 and *S. lycopersicum* cv. IRAT L3. Partial resistance against different strains of *C. michiganensis* subsp. *michiganensis* was identified in the wild relatives of the cultural tomato *Lycopersicon hirsutum – Lycopersicon entryion* (LA) 407. Resistance in LA407 was determined in population lines obtained by reverse crossing (IBC) BC2S4 in greenhouse and field conditions. Two lines of the IBC population, in particular IBL 2353 and IBL 2361, have been identified as sources that maintain high resistance at the genetic level with theoretical homology of the genome *L. esculentum* 87.5% (Francis et al., 2001).

For a long time the race R0 *P. syringae* pv. *tomato* was successfully controlled by the gene *Pto1*, resistance against which was overcome by race R1, which was discovered in 1982 in Canada. There are currently no commercial varieties of tomatoes that are resistant to the R1 race, although some wild tomato species have such genetically determined resistance. The presence of high level of resistance of isolates to California race R1 in the studied tomato lines was shown (Stamova, 2009). The disease spread index (DSI) of the resistant lines ranged from 1.00 to 1.93 on a five-point scale depending on isolate virulence. At the same time, the DSI of susceptible control varieties Chico III and ONT 7710 ranged from 4.70 to 5.00. The level of resistance of F1 plants was equal to the resistance of the maternal line.

Tomato lines with fruits different from the traditional red color were studied in order to find sources of resistance to races R0 and R1 *P. syringae* pv. *tomato* (Ganeva and Bogatzevska, 2017). Lines L1078 and L1083 with brown-red (black) fruits and L1130 with purple-red fruits were highly resistant against races R0 and R1. It was found that two lines with pink fruits L1088 and L584, which are resistant against the race R1 *P. syringae* pv. *tomato*, can be used in combined and heterosis breeding for breeding varieties of tomatoes resistant to bacterial mottle.

In order to create resistant varieties and hybrids of vegetable crops against bacterial pathogens, the use of cell selection methods is an alternative. Joint cultivation of plants with phytopathogens has become one of the effective means of plant breeding for resistance against bacterial diseases (Kolomiiets et al., 2017a). In cell selection, various

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plant cells and organs are used, as well as types of selective agents, which under optimal conditions can trigger a cascade of reactions to the pathogen similar to the whole plant. A plant or its tissue or organ, surviving under the pressure of selective assortment, is a potential source of resistance/ tolerance (Kolomiiets et al., 2017a).

In order to reduce the time of selection of tomato genotypes resistant against bacteriosis pathogens, we have developed a biomethod, which is based on the use of *in vitro* cultures of plant cells and tissues. It has been used to test the stability of 16 determinant varieties of tomatoes of Ukrainian selection. In our research, it was proven that tomato varieties Chaika, Klondike and Zoreslav are resistant against pathogens of bacterial cancer, mottle, and spotting; Flandriia, Lehin – against bacterial spotting, and Oberih, Atlasnyi, Hospodar and Kimmeriets – against bacterial mottle (Kolomiiets et al., 2017a). The selected promising genotypes can serve as a starting material for the creation of tomato varieties with high resistance against bacterial diseases.

Consequently, genetic resistance is gaining significant practical interest in the integrated biocontrol of bacterial diseases to reduce crop losses of vegetable crops. Genetic diversity, high mutation capacity and overcoming the genetic barriers of the pathogen are challenges for breeders in creating varieties that are resistant to bacterial diseases. An alternative strategy is to use partially resistant sources that involve several non-specific genes.

Genetically-modified (GM) plants are a priority in program for growing bacterial-resistant vegetable crops in many countries. However, efforts to obtain GM-resistant plants have been slowed down by complex resistance genetics and variable pathogen races (Horvath et al., 2012).

In the case of bacterial pathogens, the study of genes and mechanisms of pathogenesis and natural or induced plant resistance and parallel work with antibacterial proteins of different origins have become the fundamental basis for the implementation of molecular approaches, in particular the involvement of GM plants for the cultivation of new resistant forms. They are divided into three main categories: 1) the introduction of bacterial genes for avirulence, 2) inclusion of bacterial resistance genes against bacterial phytotoxins, and 3) expression of antibacterial proteins of plants, insects or bacteriophages as bactericidal or bacteriolytic agents (Horvath et al., 2012).

The first category includes several types of *avr*-genes that control the synthesis of race-specific elicitor (*Table 1*) (Singh et al., 2012). In order to increase plant resistance against phytopathogens, special attention is drawn to the use of genes responsible for pathogen recognition and signal transduction (R-genes) according to the concept of "gene for gene" (Singh et al., 2012), which is widely used in genetic programs of vegetable crops mainly due to the acquisition of plant full resistance to a particular pathogen. However, a significant disadvantage of the system is exceptional racial specificity. Resistance of plants is achieved due to the development of necrosis at the site of the lesion, as a result of which the infection does not get further spread. Necrosis induction requires the presence of signal peptide genes in the pathogen and the corresponding receptor in plants, the interaction of which is the trigger for the induction of hypersensitivity reaction (Khaliluev and Shpakovski, 2013).

To date, most of the known resistance genes have been characterized, in particular coding proteins with nucleotide binding site (NBS) and the region of leucine-rich repeats (LRR), which are the most abundant among cloned R-genes. Due to the established molecular structure and biochemical functions of encoded proteins, R-genes

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of tomato plants are divided into four classes: TNL, CNL, RPL and mixed (Kopfmann et al., 2016; Martins et al., 2016) (*Table 2*).

Table 1. *The bacterial genes for avirulence*

Pathogen	Genes for avirulence
Pseudomonas syringae pv. *tomato*	*avrD (avrPtoA1), avrE (avrPtoE1)* *avrPto (avrPtoC1), avrRpt2 (avrPtoB1)* *avrBs1, avrBs2* *avrBs3, avrBsT* *avrBsP (avrBs3-2)*
Xanthomonas vesicatoria	*avrRxv, avrb7* *avrB101, avrBln* *avrB102, avrBn*

Table 2. *Resistance genes of tomatoes against bacterial pathogens*

Class	Resistance genes	Pathogen
TNL (TIR-NBS-LRR)	*Bs4* *Bs2*	*Xanthomonas campestris*
	Rx3	*Xanthomonas campestris*
CNL (CC-NBS-LRR)	*Prf*	*Pseudomonas syringae*
Mixed	*Pto*	*Pseudomonas syringae*

Gene of *Bs4* NBS-LRR class contains TIR-domain, encoding aminoterminal sequences with homology to the cytoplasmic regions of the receptor protein and the IL-1 receptor of mammals and provides for the formation of tomato resistance against *X. campestris* (Khaliluev and Shpakovski, 2013). Similarly, the resistance gene *Bs2* of pepper specifically recognizes and provides resistance against strains *X. vesicatoria*, which contains the corresponding bacterial avirulent gene *avrBs2*. It is proved that the presence of the gene *Bs2* in sensitive line VF 36 reduces the progression of the disease to very low levels, and VF 36 plants have the lowest percentage of the disease among the tested varieties and the commercial tomato lines. The yield of commercial fruits from GM lines was by 2.5 times higher than that of parents, which ranged from 1.5 to 11.5 times depending on weather conditions and infectious background of the disease (Horvath et al., 2012).

The second class includes genes encoding proteins, in which there is a spiral-twisted domain. Representatives of this class are genes that cause the formation of high resistance of tomato plants against *P. syringae* (*Prf*).

Another example of an R-gene is a gene *Pto*, encoding the intracellular serine/threonine specific protein kinases, which was isolated from wild species of tomato plants *S. pimpinellifolium* L. A gene *Pto* causes resistance of plants against *P. syringae* pv. *tomato* and expresses genes of avirulence *avrPto* and *avrPtoB* (Pedley and Martin, 2003). Transfer of cDNA of the gene Pto into susceptible varieties of tomato Moneymarker and Urfa-2 caused the development of resistance against bacterial spotting of fruits (Khaliluev and Shpakovski, 2013).

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The second category comprises the toxin-antitoxin (TA) system which is localized in plasmids and chromosomes of bacteria and contains dicistronic operons, encoding two small genes, one for the toxic component and the second – for antitoxin (Kopfmann et al., 2016). Most of the toxins are endoribonucleases that operate in free or ribosome associated mRNA, the others are focused on DNA-gyrase (CcdB, ParE), tRNA-synthetase (HipA), EF-Tu (Doc) and peptidoglycan predecessors (Martins et al., 2016).

In the third category, genes of hydrolytic enzymes that degrade the cell walls of phytopathogenic bacteria were used to create GM plants with high resistance against pathogenic bacteria. For this purpose, they are used genes of β-1,3-glucanase and inducible genes of plant chitinase with lysozyme activity (PR-2 and PR-3 families of protective proteins) (Goyal and Manoharachary, 2014).

Expression of heterologous plant genes of PR-proteins and antimicrobial peptides (AMP) is the most used genetic engineering concept to improve the degree of plant resistance against diseases of bacterial nature (*Table 3*).

Table 3. *Expression of antibacterial proteins of plants and antimicrobial peptides*

Family	Gen	Resistance
PR-1	*CABPR1*	*Pseudomonas syringae* pv. *tomato*
PR-2	*GLU*	*Ralstonia solanacearum*
PR-5	*thauII*	*Xanthomonas vesicatoria*
PR-12	*alfAFP*	*Ralstonia solanacearum*
PR-13	*Thi2.1*	*Ralstonia solanacearum*
PR-14	*LjAMP1*	*Ralstonia solanacearum*
Snakins	*SN2*	*Clavibacter michiganensis* subsp. *michiganensis*

According to the modern classification, 17 families of PR-proteins and 9 – of AMP are distinguished by similarity of amino acid sequences, biochemical characteristics, biological activity and cell localization (Khaliluev and Shpakovski, 2013). Protective proteins and peptides are relatively small in size, which are positively charged and contain a significant amount of cysteine residues, which stabilizes their tertiary structure by the formation of disulfide bonds (Rahnamaeian, 2011). It was found that the majority of protective PR-proteins and AMP in micromolar concentrations have high antibacterial activity (Edreva, 2005; Van Loon, 2006).

To increase the resistance of tomato plants against pathogens promising was the use of taumatin proteins, which are isolated in the family PR-5. In research (Korneeva et al., 2008), integration into the genome of a gene *thau II*, encoding a native super sweet protein thaumatin, allowed obtaining theGM lines, which have essentially high resistance against the pathogen of tomato black bacterial spot (Korneeva et al., 2011).

Antimicrobial peptides include cecropins, magainins, sarcotoxin IA and tachyplesin I. From potato tubers they were selected antimicrobial peptides (SN1, SN2) with a unique amino acid sequence, which are assigned to individual families AMP – snakins (Mohan, 2011). Their distinctive feature is the presence in the amino acid sequence of the site, which is characteristic of the hemolytic venom of snakes. Snakin SN2 super-production in GM tomato plants contributed to a significant delay and reduction in symptoms of the development of bacterial cancer when compared to the control (Khaliluev and Shpakovski, 2013; Balaji and Smart, 2012).

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Using the method of agrobacterial transformation of the obtained tomato plants with the gene of a synthetic analogue of magainin II (MSI-99) – AMP, which is isolated from the skin of the African clawed frog (*Xenopus laevis*) (Alan et al., 2004). According to the results of plant testing for resistance against *P. syringae* pv. *tomato* it was found that the degree of symptoms of the disease in some lines was much less than in the control.

Vector construction pBI121-spCB, as part of the T-DNA of which it is localized natural gene of cationic lytic cecropin peptide B, isolated from the silkworm (*Hyalophora cecropia*), and with the signal sequence of the gene of α-amylase of barley used to produce the GM tomato plants resistant against bacterial wilt and bacterial black spotting (Jan et al., 2010).

It can be concluded that there is still no reliable information on the production of GM tomato plants that are resistant to bacterial diseases and suitable for commercial use. The complexity of obtaining such plants is explained by the genetic complexity and versatility of this feature, as well as the rapid loss of plant resistance acquired. It is predicted that the partial overcoming of the existing problems is expected by embedding into the genome of plants of simultaneously several genes of different families, protein products of which have different mechanisms of action (Khaliluev and Shpakovski, 2013).

Summary

It is shown that agrotechnical measures are clearly preventive in nature, while chemical and antibiotic use are low effective, showing side effects, in particular, phytotoxicity and spread of resistant strains of pathogens. It was confirmed antagonistic activity of bacteria of genera *Bacillus* and *Streptomyces* to phytopathogenic bacteria, and biopreparations based on them are recommended for biocontrol of bacterial pathogens. Effective and proved measures are the cultivation of resistant varieties, the selection of which is based on the involvement of wild species as natural sources of resistance, and GM vegetable crops containing avr-genes, genes of resistance against bacterial phytotoxins, PR-proteins and AMP.

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