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Crown gall of grapevine and prospects for its biological control

Abstract. The relevance of this study is conditioned by the spread of bacterial diseases of grapes in the south of Ukraine and the necessity of improving methods of pathogen identification and protection. The purpose of this study was to establish the area of bacterial grape cancer in Odesa

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region and to develop preventive measures based on the biological method. The study involved the inspection of industrial plantations for symptoms of the disease and its spread in the field. The molecular biological method of real-time polymerase chain reaction (PCR) was employed to identify the crown gall pathogen. The study was conducted following a certified methodology. PCR laboratory equipment was used to identify phytopathogens. As a result of the phytosanitary inspection of grape plantations of various farms in Odesa region, the study found grape bushes with characteristic symptoms of crown gall, with tumorous tissue growths in various parts of the plants: on the branches, stem, and grafting sites. Overall, grape crown gall is characterised by a wide distribution in the study area. According to the findings of the phytosanitary survey, the spread of bacterial grape cancer on different varieties ranged between 0.3-35%. The causative agent of the disease, *A. tumefaciens*, was identified in real time by PCR. The disease manifested itself in the form of characteristic symptoms and developed in a latent form. The developed multiplex PCR allowed for the simultaneous analysis of multiple strains of pathogenic agrobacterial isolates. Two isolates of agrobacteria were identified among the majority of isolates that were subsequently tested for tumour suppression: ILVM1 and ILVM2, which had elevated levels of antagonistic properties against the crown gall pathogen. The pathogenicity of the isolated agrobacteria on test plants of tomato and sunflower confirmed the findings of these properties obtained *in vitro*. The isolates of ILVM1 and ILVM2 agrobacteria considerably inhibited the growth of tumours on the stems of test plants compared to the pathogenic strain, and therefore they can be used in the future against the crown gall pathogen and to protect grape plants from secondary infection by the pathogen from the soil. The findings can be used to improve methods of biological plant protection against bacterial infections

Keywords: grape diseases; polymerase chain reaction; *Agrobacterium tumefaciens*; antagonist bacteria; biological protection

INTRODUCTION

Crown gall of grape, caused by *Agrobacterium tumefaciens* (Smith et Townsend 1907) Conn 1942, is a systemic, chronic disease. There is almost no means of protection against it, and control is mainly based on the prevention of plant damage, prompt diagnosis and identification of the pathogen. The disease causes a 10-20% decrease in yield and an average 15-20% decrease in sugar content (Sawant, 2023). It also impairs vine ripening. The factors contributing to plant damage include frost damage, mechanical damage, infection of planting material, soil infection, and high rates of disease transmission. As a result of the development of crown gall, industrial grape growing technologies may face difficulties in obtaining high-quality products.

As indicated by Y. Noutoshi *et al.* (2020) and I.A. Kovaleva *et al.* (2022), fungal, viral, and bacterial diseases are widespread and harmful in various grape growing regions. Among the latter, crown gall is characterised by high harmfulness in vineyards. This disease can spread rapidly among plants, especially under favourable conditions for bacteria, which significantly complicates the fight against it. As a result of crown gall, the overall productivity of vineyards and the quality of the crop are reduced, which leads to serious economic losses. The study by A. Konup *et al.* (2019) found that among the various infectious diseases in the vineyards of certain districts of Odesa region, crown gall accounts for about 25%. These statistics highlight the relevance of the problem and the importance of developing effective measures to control and treat this disease. Furthermore, the high prevalence of crown gall in the region requires attention from plant protection specialists and researchers to monitor the condition of vineyards and take prompt action. According to M.G. Thompson *et al.* (2020), symptoms of the disease are more likely to occur on bush boles, especially after damage by abiotic factors. This suggests that stressful conditions, such as drought, excessive humidity, or temperature fluctuations, can substantially weaken plants, making them more vulnerable to infections. Therefore, monitoring

environmental conditions and their impact on the condition of grape bushes is a crucial aspect in preventing the development of crown gall. T. Nguyen-Huu *et al.* (2020) noted that crown gall weakens grapevines, causes a sharp decline in yield, and in some cases leads to partial or complete plant death. This highlights the need for prompt diagnosis and treatment of the disease, as a delay in its detection can lead to major economic losses for viticulturists. Additionally, agronomic measures to strengthen plants, such as improved nutrition and water management, can help reduce the damage caused by crown gall. T. Meyer *et al.* (2019) also found that the danger of crown gall is that the bacterium, accumulating in the soil, can later cause root infection in new plantations of grape plants.

Notably, bacterial plant diseases are generally challenging to control. According to L.M. Yepes *et al.* (2019), copper bactericides and antibiotics are ineffective, while disease prevention by planting pathogen-free planting material is complicated by the vegetative propagation of infected, asymptomatic vines. Under such conditions, according to A.T. Wong *et al.* (2021), it is promising to use non-pathogenic strains of Rhizobium (synonym for Agrobacterium) by co-inoculation with pathogenic bacterial isolates to reduce the damage to grapevine seedlings.

The purpose of this study was to conduct a phytosanitary inspection of grape plantations in the Odesa region to determine the spread of crown gall and identify the causative agent. For this purpose, it was necessary to extract and screen agrobacterial isolates for their antagonistic properties against *A. tumefaciens.*

MATERIALS AND METHODS

The study was conducted at the Laboratory of Virology and Microbiology of the National Scientific Centre "Institute of Viticulture and Winemaking named after V.E. Tairov" of the National Academy of Agrarian Sciences (NAAS) of Ukraine. The study followed the ethical standards set out in the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973). The grape plantations were inspected at the research farms of the National Scientific Centre "Institute of Viticulture and Winemaking named after V.E. Tairov" and industrial farms in Odesa region according to DSTU 3355:1996 (1997) and DSTU 4390:2005 (2006). Visual phytosanitary inspection and assessment of the degree of crown gall infection was based on the examination of plants in the plantations. Samples of grape plants of Cabernet Sauvignon, Merlot, and Chardonnay varieties were selected based on external symptoms: the presence of tumours in certain areas of the plants, as well as asymptomatic plants to detect the latent form of the disease. The grape plant samples were collected, stored, and prepared according to ISO 16578:2022 (2022). Classical polymerase chain reaction (PCR) was used to identify *A. tumefaciens*.

The study employed the method of semi-selective nutrient media for the determination of agrobacteria, original and modified methods for the isolation of DNA of the crown gall pathogen, and PCR for the identification of *A. tumefaciens*. Bacteria were isolated from grape tissues according to the Lehoczky method (Lehoczky, 1971). Tumour, ripened vine, strands, or roots were used as samples for detection of the crown gall pathogen. The average sample of vines was formed from 1-3 two-year-old shoot fragments from several different parts of the bush, which were selected as close to the perennial wood as possible. For the extraction of agrobacteria, discs 0.2-0.5 cm tall were cut from each fragment; 15-20 discs of the average sample were placed in 3-5 ml of sterile distilled water. The agrobacteria were isolated from the samples under aseptic conditions using sterile dishes, instruments, and culture media. Agrobacteria were isolated on semi-selective nutrient medium (Roy and Sasser medium) of the following composition per 1,000 ml: $MgSO_4$ – 0.20 g, K_2HPO_4 – 0.9 g, KH_2PO_4 – 0.7 g, adonitol – 0.4 g, yeast extract – 0.14 g, NaCl – 0.2 g, boric acid – 1.0 g, agar – 15 g, pH – 7.2. After autoclaving and cooling to +50°C, the following were added: triphenyltetrazolium chloride – 0.08 g, trimethoprim – 0.02 g, d-cycloserine – 0.02 g, cycloheximide – 0.25 g. The colonies of agrobacteria obtained on the medium in Petri dishes were inoculated into tubes with bevelled agar and incubated for 1 day in a thermostat at +28°C. Subsequently, isolates of agrobacteria were identified by PCR (Abolmaaty *et al.*, 2000). The plasmid

DNA of *A. tumefaciens* was isolated using cetavlon, thermal method or alkaline lysis, and the resulting preparations were stored at −20°C. For PCR, reagents qualified as "for molecular biology", sterile solutions, and glassware were used. PCR was performed following the certified methodology No. MVV 002-85/3 – 2006.

To detect *A. tumefaciens*, specific primers were used for the ipt isopentenyltransferase gene: ipt1/ipt2 GATCG(G/C)GTCCAATG(CT)TGT/GATATCCATCGATC(TC)CTT and for the VirD2 endonuclease gene of the pathogenicity plasmid: virD2A/virD2C ATGCCCGATCGAGCTCAAGT/TCGTCTGGCTGACTTTCGTCATAA (Haas *et al.*, 1995). Primers were synthesised by Fermentas (Lithuania). Real-time PCR was performed using forward and reverse primers, fluorescently labelled DNA probes, reaction mixture in the amount of 20 µl (H_2O_2 – 8.5 µl; 10×PCR buffer – 2.5 µl; 4mM dNTP – 2.5 µl (1.76 mM – 2.84 µl); pr1 – 0.5 µM; pr2 – 0.5 µM; fluorescent probe – 0.1 µM, Taq polymerase (2.5 u/µl) (*Pfu DNA, Fermentas, Lithuania*) – 0.25 µl; Mg^{2+} – 3.0 mM and 5 µl of NCS, or PCS, or internal control, or test sample (at the bottom of the tube). The concentrations of forward and reverse primers and fluorescent DNA probes were selected empirically. To control the reaction, the study used a negative control sample (NCS) – 1×PCR buffer and a positive control sample (PCS) – the reference strain Fa-2 of the crown gall pathogen. Amplification was performed in a programmable thermocycler Rotor-Gene 6000 (Corbett Research Pty Ltd., Australia), which provided a temperature range within +40-100 °C, in the following mode:

1. Denaturation 95°C – 30 sec;
2. Annealing 60°C – 1 min;
3. Elongation – 72°C – 1 min (30 cycles);
4. Final elongation – 72°C – 10 min.

The solution with the amplification products was stored at −20°C. Initial denaturation was 95°C for 12 min. Fluorescence signal accumulation was measured for 4 channels, respectively: FAM/Green (470 nm/510 nm) for the identification of the crown gall pathogen, JOE/Yellow/HEX (530 nm/555 nm), ROX/Orange (585 nm/610 nm), Cy5/Red (625 nm/660 nm, and Cy3.5/Orange (585 nm/610 nm) for the endogenous internal control signal. The analysis results were recorded, and threshold cycles were calculated using Rotor-Gene 6000 Series Software 1.7. The amplification products were detected by increasing the fluorescence of the intercalating dye when it forms a complex with the double-stranded DNA of the crown gall pathogen. A positive sample was considered the one that showed an increase in the fluorescent signal on one of the colour channels of the amplifier.

To determine the pathogenicity of the extracted agrobacteria, test plants were used: tomatoes (*Solanum lycopersicum* L.) and sunflower (*Helianthus annuus* L.). The plants were inoculated with a suspension of these agrobacteria in the amount of 10^9 CFU/ml by cutting the internode of the stem at the level of 2-3 leaves. The wound was covered with Parafilm film (USA) to prevent drying of the bacterial suspension. The inoculated plants were incubated for 14-28 days at +25°C. Uninfected plants inoculated with sterile distilled water were used as a negative control. The positive control included plants inoculated with the reference *A. tumefaciens* pathogenic strain Fa-2 (isolated from grape roots; kindly provided by Dr T.J. Burr, USA).

Infection of plants by pathogenic bacteria *A. tumefaciens* occurs in the soil through damaged roots. To investigate the antagonistic effect of non-pathogenic isolates of *A. tumefaciens* ILVM1 and ILVM2, the cut roots of grape seedlings were soaked for one hour in a cell suspension (10^9 CFU/ml). After soaking, the treated grape seedlings were planted in pots with soil inoculated with a mixture of three strains of *A. tumefaciens*: Fa-2, 271, and IDI with a final concentration of 10^8 CFU/g soil and were grown in greenhouses. The development of tumours formed on the roots and stems of plants was studied.

RESULTS AND DISCUSSION

During the inspection of plantations in the industrial viticulture zone in the Odesa region, tumours typical of crown gall were found on grape bushes (Fig. 1). The tumours were identified by PCR analysis. The latent infection with the crown gall of grape pathogen on individual grape varieties ranged within 0-30%, depending on the susceptibility of the variety and the origin of the planting material.

Figure 1. Symptoms of crown gall on the grape plant
Source: photograph taken by the authors of this study

In studies, symptoms of the disease were observed on seedlings with latent infection as early as 1-2 years after planting. With the affected planting material, the crown gall pathogen enters the soil, and the infected area can continue to be a reservoir of *A. tumefaciens* for a long time.

A visual inspection of the vineyards in the study area revealed the presence of varying degrees of crown gall on young and fruiting plantations. The degree of development of the disease on technical varieties was as follows: Cabernet Sauvignon – up to 35%, Merlot – 25%, Chardonnay – 15%, which suggests the need to develop and improve crown gall control measures. Y. Wang *et al.* (2018) found that all grape varieties may be at risk of infection with the crown gall pathogen to some extent, and there are no varieties entirely resistant to this pathogen.

The use of antagonist microorganisms as part of a biological method of disease control is promising. Specifically, in the studies of H. Pan *et al.* (2022), the *Bacillus megaterium* L2 strain exhibited antibacterial activity against *A. tumefaciens* T-37, as its biologically active substances can destroy the integrity of the cell membrane, damage cellular structures, affect cellular metabolism, and inhibit protein synthesis. In the study by G.E. Qing *et al.* (2024), *Bacillus amyloliquefaciens* strain KN-527 had an inhibitory effect on *Agrobacterium vitis* in the early stages of crown gall development. At the same time, it is also interesting to use other isolates of agrobacteria against grape crown gall.

To search for antagonist bacteria, 50 bacterial isolates were isolated from soil in vineyards. The grown bacteria had a characteristic pink-red colour with a white border when cultivated on Roy and Sasser medium. The pathogenicity of the agrobacterium isolates was tested by real-time PCR (Fig. 2).

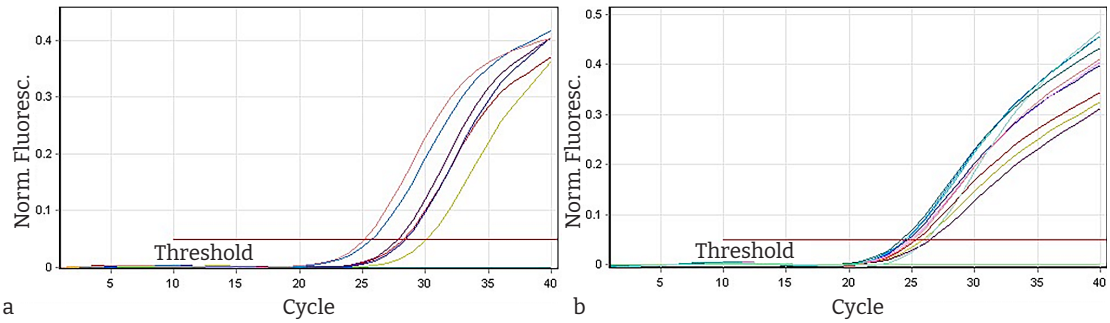

Figure 2. Results of testing isolates of agrobacteria for pathogenic properties
Note: a – bacterial isolates recovered from the soil in the vineyard aisles; b – bacterial isolates from the rhizosphere of grape bushes: 1 – positive control sample (PCS), reference strain Fa-2; 2 – negative control sample (NCS), PCR buffer, curves – pathogenic isolates
Source: developed by the authors of this study

It was found that most of the agrobacteria isolated from samples of grape plants collected in the vineyards of Odesa region contained fragments of *virD2* and *virF* genes simultaneously, which indicates their high virulence. To determine the antagonistic properties, non-pathogenic agrobacteria were used, which showed a zone of growth retardation of the test object at

the level of 17-22 mm on the nutrient medium (Fig. 3). The screening for antagonistic properties revealed two isolates of *A. tumefaciens* ILVM1 and *A. tumefaciens* ILVM2, which had an elevated level of *in vitro* activity.

Screening tests for the presence of antagonistic properties of agrobacterial isolates on test plants (*S. lycopersicum, H. annuus*) confirmed the results of determining these properties obtained *in vitro*. It was found that most of the isolates tested for tumour inhibition had minor antagonistic properties and suppressed the disease by 20-30% compared to the positive control, but isolates of agrobacteria ILVM1 and ILVM2 considerably inhibited tumour growth on the stems of test plants (96%) compared to the pathogenic strain Fa-2 (Fig. 4; 5).

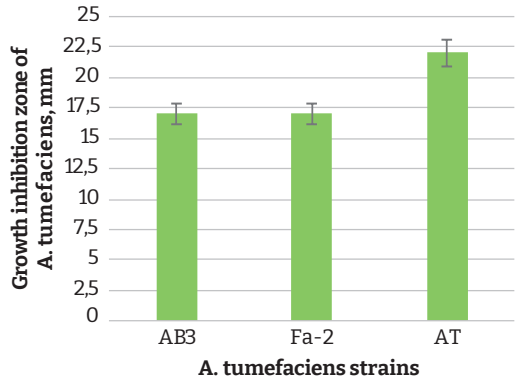

Figure 3. Antagonistic activity of a non-pathogenic isolate of ILVM 1 against reference plant pathogenic strains of *A. tumefaciens*
Source: developed by the authors of this study

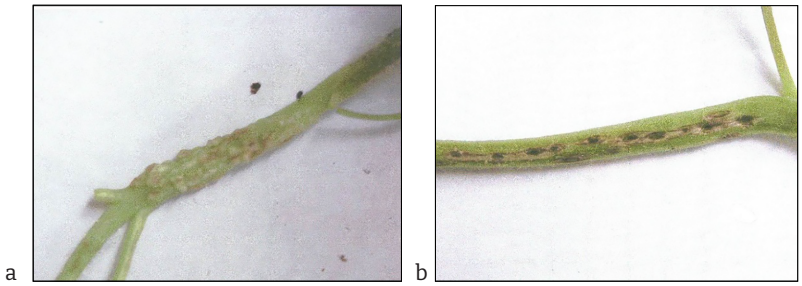

Figure 4. Influence of *A. tumefaciens* bacterial isolates on the manifestation of crown gall on *S. lycopersicum*
Note: a – disease symptoms after inoculation of the test plant with a suspension of bacteria of the *A. tumefaciens* pathogenic strain Fa-2 (10^9 CFU/ml); b – no disease symptoms after joint inoculation of the *S. lycopersicum* stem with a suspension of a mixture of non-pathogenic isolate ILVM1 and *A. tumefaciens* pathogenic strain Fa-2 (10^9 CFU/ml)
Source: photograph taken by the authors of this study

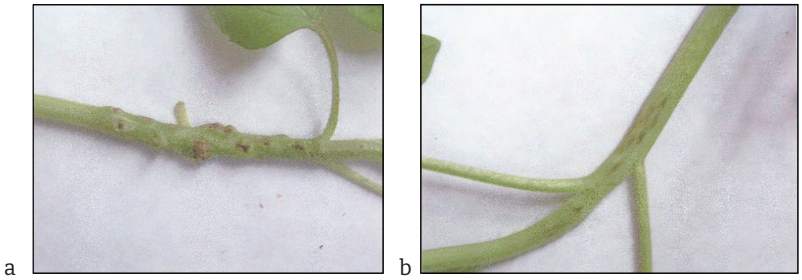

Figure 5. Effect of *A. tumefaciens* bacterial isolates on the manifestation of crown gall of *H. annuus*
Note: a – disease symptoms after inoculation of the test plant with a suspension of the *A. tumefaciens* pathogenic strain Fa-2 (10^9 CFU/ml): b – no disease symptoms after inoculation of the *H. annuus* stem with a suspension of a mixture of non-pathogenic isolate ILVM2 and *A. tumefaciens* pathogenic strain Fa-2 (10^9 CFU/ml)
Source: photograph taken by the authors of this study

The results of the experiment were recorded 30 days after inoculation. It was found that in the places of inoculation of the stems of test plants *S. lycopersicum* and *H. annuus* with the *A. tumefaciens* pathogenic strain Fa-2, characteristic tumours appeared (Fig. 4a and 5a), which were not observed on test plants inoculated with a suspension of a mixture of non-pathogenic bacterial isolate ILVM2 and *A. tumefaciens* pathogenic strain Fa-2 (Fig. 4b and 5b).

The authors of the present study believe that the absence of symptoms of crown gall in test plants treated with a mixture of pathogenic and antagonist bacterial isolates is caused by the inhibition of *pehA* gene expression, which ensures the virulence of *A. tumefaciens*. It is probable that this process inhibits the transformation of host cells, thereby preventing tumour formation. At the same time, this hypothesis requires more detailed molecular biological research.

Treatment of the roots of different grape seedling varieties with ILVM1 isolate resulted in a 53-86% reduction in the percentage of plants with disease symptoms. The greatest effect was observed on Chardonnay plants (Fig. 6).

The efficacy of ILVM2 isolate was slightly higher and amounted to 66-87% in the varieties under study (Fig. 7). Thus, it was confirmed that preliminary inoculation of grape seedling roots with isolates of ILVM1 and ILVM2 was effective in reducing the damage to the root system by the crown gall pathogen.

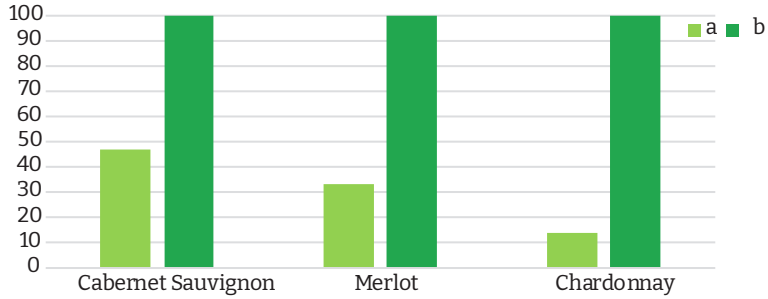

Figure 6. The effect of treatment of grape roots of different varieties with ILVM isolate on the susceptibility of seedlings to crown gall

Note: a – number of plants, %, affected by *A. tumefaciens* after treatment with ILVM1; b – control, number of plants, %, affected by *A. tumefaciens* without treatment with non-pathogenic isolate

Source: developed by the authors of this study

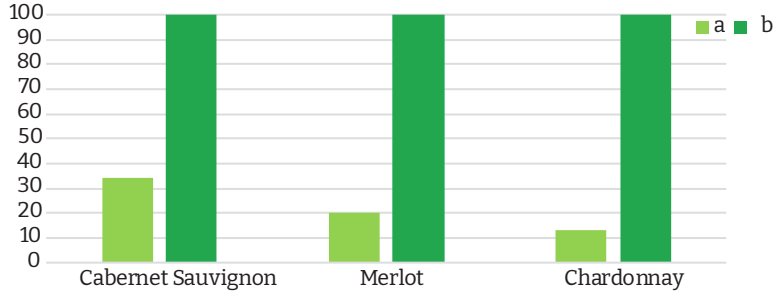

Figure 7. The effect of treatment of grape roots of different varieties with ILVM 2 isolate on the susceptibility of seedlings to crown gall

Note: a – number of plants, %, affected by *A. tumefaciens* after treatment with ILVM1; b – control, number of plants, %, affected by *A. tumefaciens* without treatment with non-pathogenic isolate

Source: developed by the authors of this study

The positive results obtained in the laboratory prompted field trials of the antagonistic effect of ILVM1 and ILVM2 isolates. These studies are a vital part of recent developments, especially in the search for biological defence products. Although positive results may be obtained in laboratory experiments, field trials often fail to produce the expected results, which necessitates such experiments.

To protect grape seedlings from secondary infection by pathogenic agrobacteria that are soilborne, the roots were treated with a mash of clay and a mixture of ILVM1 and ILVM2 isolates at a concentration of 10^9 CFU/ml. The effectiveness was evaluated in a year. It was found that in the variants with a mixture of non-pathogenic isolates of agrobacteria, the number of plants affected by *A. tumefaciens* ranged within 10-27%, which suggests a decrease in the spread of crown gall by 73-90%, depending on the grape variety (Fig. 8). The analysis showed the absence of pathogenic isolates of agrobacteria in the rhizosphere soil.

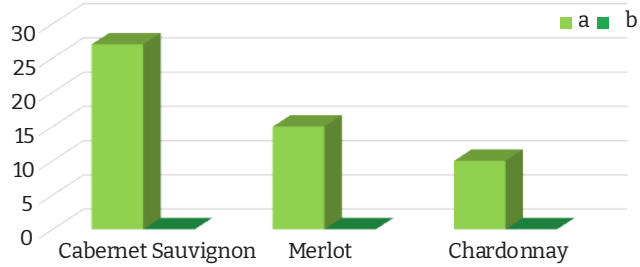

Figure 8. Influence of combined use of agrobacterium antagonist isolates on the presence of crown gall pathogen in grape plants of different varieties and in rhizosphere soil

Note: a – number of plants, %, affected by *A. tumefaciens* after treatment of seedlings with ILVM1 + ILVM 2 mixture; b – presence, %, of *A. tumefaciens* in the rhizosphere after treatment of seedlings with ILVM1 + ILVM2 mixture

Source: developed by the authors of this study

The unique action of *A. tumefaciens* lies in the possibility of its use as a tool during plant reproduction (Ben Abdallah *et al.*, 2015). The use of an agrobacterium not only shortens the conventional process of plant propagation, but also allows the introduction of completely new genes into the plant, which are not typical for the plant, through genetic engineering (Lacroix & Citovsky, 2019; Guo *et al.*, 2019).

According to T. Tzfira *et al.* (2017), *A. tumefaciens* is one of the most interesting and important bacteria for the detailed investigation of its antagonistic properties. It can be used to control crown gall. This is the first and most successful example of biological protection of grapes from a disease (Frikha-Gargouri *et al.*, 2017).

Other scientists conducted comparable studies on biological control of the disease. V. Stockwell *et al.* (1996) found that *Agrobacterium radiobacter* strain K84 controls the disease caused by *A. tumefaciens* by producing the plasmid agrocin 84, an antibiotic with specific activity against certain strains of the pathogen. In S. Chopra *et al.* (2016), several non-pathogenic strains also helped to reduce infection, but especially *A. radiobacter* K84, which completely prevented the disease when added to the wound sites at a 1:1 ratio with *A. tumefaciens* cells.

According to R. Peñalver *et al.* (2000), *Agrobacterium radiobacter* strain K84 has been a biological control agent for crown gall for many decades worldwide. Despite its demonstrated efficacy, the greatest risk of failure with the K84 strain is the possibility of transferring the pAgK84 plasmid to pathogenic Agrobacterium strains. Later, the second generation of the K84 strain was obtained and the genetically modified strain was named K1026. It contains a deletion in the pAgK84 transfer region. A considerable amount of research has also been conducted to compare the two strains in terms of their ability to control disease, plasmid transfer, antibiotic production, root colonisation, and rhizosphere survival.

Q. Wei *et al.* (2009) indicated that *Agrobacterium vitis* E26 is a promising potential biocontrol agent for crown gall of grapevine. However, its safety and environmental risks should be understood. According to the findings of the study, the researchers found that the *A. vitis* E26 strain does not contain the virA and virG determinants. This suggests that this strain is unlikely to cause symptoms in both host and non-host plants.

Y. Hao *et al.* (2011) found that the avirulent strain of *A. tumefaciens* D3 contains a putative structural gene for ACC deaminase (acdS) and a regulatory gene (acdR = lrpL). Studies revealed that under gnotobiotic conditions, wild-type *A. tumefaciens* D3 can promote root elongation, while the acdS and lrpL double mutant *A. tumefaciens* strain D3-1 lost this ability. When co-inoculated with the virulent *A. tumefaciens* strain C58, both *A. tumefaciens* D3 wild type and *A. tumefaciens* D3-1 mutant were found in damaged castor oilseed rape plants, which can substantially suppress the development of crown gall caused by *A. tumefaciens* C58.

A. Kawaguchi *et al.* (2017) identified a non-pathogenic *Agrobacterium vitis* strain ARK-1 and found it to be effective in suppressing tumour formation on grapevines. Further studies of the *Rhizobium vitis* ARK-1 strain in the field showed its therapeutic effect and a decrease in the number of grape plants with symptoms of crown gall. According to A. Kawaguchi *et al.* (2019), co-inoculation of *R. vitis* ARK-1 with the pathogenic strain Ti VAT03-9 in a 1:1 cell ratio in grapevine shoots resulted in a significantly lower expression of the virulence genes virA, virD3, and virG of VAT03-9.

A. Wong *et al.* (2021) tested the non-pathogenic *R. vitis* strain ARK-1 (isolated in Japan) against four pathogenic *R. vitis* isolates from grapevines in Virginia (USA). Specifically, co-inoculation with ARK-1 significantly reduced the occurrence of disease symptoms and the average size of growths, except for the ACME15 isolate.

Scientists are also investigating other areas of biological protection of grapes against crown gall. D. Ferrigo *et al.* (2017) conducted experimental trials to assess the effectiveness of treatments based on non-pathogenic endophytes. Two-year *in planta* trials conducted on rootstocks treated with endophytic isolates showed the effectiveness of two bacterial endophytes from the genus Curtobacterium and a fungal isolate from the genus Acremonium. They led to a reduction in the development of disease symptoms. The researchers also noted the effectiveness of commercial biological control agents based on *B. subtilis* SR63 and *T. asperellum* T1 strains against *A. vitis*.

In Ukraine, N. Lemanova & M. Magher (2019) investigated the biological preparation "Paurin" based on the bacteria *Pseudomonas fluorescens* CNMN-PsB-04. For this, the biological preparation was used by treating apple MM-106 rootstock cuttings and fruit seeds before planting them in the soil. This method helped to reduce the damage to plants by crown gall by 2.0-2.5 times.

N.V. Limanska *et al.* (2019) investigated the effect of *Lactobacillus plantarum* bacteria on tumour formation and survival of *A. tumefaciens* pJZ on plant surfaces and tissues. The experimental results showed that the number of test plants (of different species) with disease symptoms in the presence of *L. plantarum* in the inoculum decreased by 85-100%. The average weight of tumours was reduced by 2-12 times compared to the control. On grapes, inoculation with a mixture of *A. tumefaciens* and *Lactobacillus* caused stimulation of plant growth, which was manifested in an increase in the number of buds that opened, an increase in the average length of shoots and an increase in the average area of the leaf apparatus.

The findings of the experiments presented in this study demonstrated a wide range of antagonistic activity of the studied isolates ILVM1 and ILVM2 against the reference agrobacterium *A. tumefaciens.* This points to the possibility of expanding the scope of practical use of these strains to create a potential effective biological product as a means of controlling the pathogen of crown gall in the south of Ukraine. In the future, the development and use of a product based on these bacteria will help reduce the percentage of plants affected by soil infection and prevent the spread of the disease, which will considerably increase the economic level of seedling production, increase the quantity, and improve the quality of the crop.

CONCLUSIONS

The study investigated the spread of crown gall in Odesa region, screened the antagonistic

activity of non-pathogenic isolates of *A. tumefaciens in vitro* and *in vivo*, and determined their ability to control the disease in the field.

According to the findings of the phytosanitary inspection of grape plantations in Odesa region, the disease prevalence ranged within 15-35% (on Chardonnay, Merlot, and Cabernet Sauvignon varieties). Using the real-time PCR method, pathogenic isolates of agrobacteria were identified, and non-pathogenic isolates were also recovered. The latter were tested for antagonistic properties against the crown gall pathogen. Thus, *in vitro* on nutrient medium, the growth inhibition zone of the test object was 17-22 mm. As a result of the antagonistic properties study, two isolates of *A. tumefaciens* ILVM1 and *A. tumefaciens* ILVM2 were identified, which had a pronounced activity level. It was proved that non-pathogenic isolates of agrobacteria ILVM1 and ILVM2 inhibited the development of crown gall *in vitro* and *in vivo* on test plants *S. lycopersicum* and *H. annuus*.

The obtained findings on plants of different grape varieties allowed establishing the effectiveness of treatment of seedling roots with isolates of non-pathogenic agrobacteria ILVM1 and ILVM2. Specifically, the ILVM1 isolate led to a 53-86% reduction in the percentage of plants with disease symptoms. The effectiveness of ILVM2 isolate was 66-87%. The lowest number of diseased plants was observed on plants of the Chardonnay variety.

With the combined use of non-pathogenic isolates of agrobacteria, the number of plants affected by *A. tumefaciens* ranged within 10-27%, which indicates a decrease in the spread of crown gall by 73-90%, depending on the grape variety.

Thus, isolates of agrobacteria ILVM1 and ILVM2 showed antagonistic properties to the pathogen of grape crown gall, which in the future allows using them to develop biological means of protection against this dangerous disease.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

REFERENCES

[1] Abolmaaty, A., Vu, C., Oliver, J., & Levin, R. (2000). Development of a new lysis solution for releasing genomic DNA from bacterial cells for amplification by polymerase chain reaction. *Microbios*, 101, 181-189.

[2] Ben Abdallah, D., Frikha-Gargouri, O., & Tounsi, S. (2015). *Bacillus amyloliquefaciens* strain 32a as a source of lipopeptides for biocontrol of *Agrobacterium tumefaciens* strains. *Journal of Applied Microbiology*, 119, 196-207. doi: 10.1111/jam.12797.

[3] Chopra, S., Palencia, A., Virus, C., Schulwitz, S., Temple, B.R., Cusack, S., & Reader, J. (2016). Structural characterization of antibiotic self-immunity tRNA synthetase in plant tumour biocontrol agent. *Nature Communications*, 7, article number 12928. doi: 10.1038/ncomms12928.

[4] Convention on Biological Diversity. (1992, June). Retrieved from https://zakon.rada.gov.ua/laws/show/995_030#Text.

[5] Convention on the Trade in Endangered Species of Wild Fauna and Flora. (1973, June). Retrieved from https://zakon.rada.gov.ua/laws/show/995_129#Text.

[6] DSTU 3355:1996. (1997). *Agriculturel products of plant. Technigue of inspection and sampling for guarantine examination and expertise*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=91446.

[7] DSTU 4390:2005. (2006). *Grape rootings and vine cuttings. Specifications*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=91479.

[8] Ferrigo, D., Causin, R., & Raiola, A. (2017). Effect of potential biocontrol agents selected among grapevine endophytes and commercial products on disease. *BioControl*, 62, 821-833. doi: 10.1007/s10526-017-9847-3.

[9] Frikha-Gargouri, O., Ben Abdallah, D., Bhar, I., & Tounsi, S. (2017). Antibiosis and *bmyB* gene presence as prevalent traits for the selection of efficient bacillus biocontrol agents against disease. *Frontiers in Plant Science*, 8, article number 1363. doi: 10.3389/fpls.2017.01363.

[10] Guo, M., Ye, J., Gao, D., Xu, N., & Yang, J. (2019). Agrobacterium-mediated horizontal gene transfer: mechanism, biotechnological application, potential risk and forestalling strategy. *Biotechnology Advances*, 37(1), 259-270. doi: 10.1016/j.biotechadv.2018.12.008.
[11] Haas, J.H., Moore, L.W., Ream, W., & Manulis, S. (1995). Universal PCR primers for detection of phytopathogenic Agrobacterium strains. *Applied and Environmental Microbiology,* 61(8), 2879-2884. doi: 10.1128/AEM.61.8.2879-2884.1995.
[12] Hao, Y., Charles, T.C., & Glick, B.R. (2011). ACC deaminase activity in avirulent *Agrobacterium tumefaciens* D3. *Canadian Journal of Microbiology*, 57, 278-286. doi: 10.1139/w11-006.
[13] ISO 16578:2022. (2022). *Molecular biomarker analysis – requirements for microarray detection of specific nucleic acid sequences*. Retrieved from https://www.iso.org/standard/80968.html.
[14] Kawaguchi, A., & Nita, M., Ishii, T., Watanabe, M., & Noutoshi, Y. (2019). Biological control agent *Rhizobium* (=*Agrobacterium*) *vitis* strain ARK-1 suppresses expression of the essential and non-essential vir genes of tumorigenic *R. vitis*. *BMC Research Notes*, 12, article number 1. doi: 10.1186/s13104-018-4038-6.
[15] Kawaguchi, A., Inoue, K., Tanina, K., & Nita, M. (2017). Biological control for grapevine using nonpathogenic *Rhizobium vitis* strain ARK-1. *Proceedings of the Japan Academy, Series B*, 93(8), 547-560. doi: 10.2183/pjab.93.035.
[16] Konup, A., Muliukina, N., & Konup, L. (2019). Detection of virus, bacterial and phytoplasmic diseases on vineyards of Odesa oblast. *Bulletin of Agricultural Science*, 4(793), 24-29. doi: 10.31073/agrovisnyk201904-04.
[17] Kovaleva, I.A., Janse, L.A., Konup, L.A., Zelenyanskaya, N.N., Vlasov, V.V., Konup, A.I., Muljukina, N.A., Kyryk, N.N., & Pikovskyi, M.Y. (2022). Detecting the infection of the cabernet sauvignon variety of clonal origin by grape viruses. *Cytology and Genetics*, 56(6), 504-512.
[18] Lacroix, B., & Citovsky, V. (2019). Pathways of DNA transfer to plants from *Agrobacterium tumefaciens* and related bacterial species. *Annual Review of Phytopathology*, 57(1), 231-251. doi: 10.1146/annurev-phyto-082718-100101.
[19] Lehoczky, J. (1971). Further evidences concerning the systemic spreading of Agrobacterium tumefaciens in the vascular system of grapevines. *Vitis,* 10, 215-221.
[20] Lemanova, N., & Magher, M. (2019). Biological method for prevention grown gall in horticulture, *Microbiological Journal*, 81(2), 36-40. doi: 10.15407/microbiolj81.02.036.
[21] Limanska, N.V., Galkin, M.B., Ivanytsia, V.O. (2019). Effect of Lactobacillus plantarum on survival of agent and tumour formation. *Microbiological Journal*, 81(1), 22-33. doi: 10.15407/microbiolj81.01.022.
[22] Meyer, T., Thiour-Mauprivez, C., Wisniewski-Dyé, F., Kerzaon, I., Comte, G., Vial, L., & Lavire, C. (2019). Ecological conditions and molecular determinants involved in *Agrobacterium lifestyle* in tumors. *Frontiers in Plant Science*, 10, article number 978. doi: 10.3389/fpls.2019.00978.
[23] Nguyen-Huu, T., Doré, J., Aït Barka, E., Lavire, C., Clément, C., Vial, L., & Sanchez, L. (2020). Development of a DNA-based real-time PCR assay to quantif *Allorhizobium vitis* over time in grapevine (*Vitis vinifera* L.) plantlets. *Plant Disease*, 105(2), 384-391. doi: 10.1094/PDIS-04-20-0732-RE.
[24] Noutoshi, Y., Toyoda, A., Ishii, T., Saito, K., Watanabe, M., & Kawaguchi, A. (2020). Complete genome sequence data of tumorigenic *Rhizobium vitis* strain VAT03-9, a causal agent of grapevine disease. *Molecular Plant-Microbe Interactions*, 33, 1280-1282. doi: 10.1094/MPMI-07-20-0180-A.
[25] Pan, H., Xiao, Y., Xie, A., Li, Z., Ding, H., Yuan, X.J., Sun, R., & Peng, Q. (2022). The antibacterial mechanism of phenylacetic acid isolated from *Bacillus megaterium* L2 against *Agrobacterium tumefaciens*. *Peer J*, 10, article number e14304. doi: 10.7717/peerj.14304
[26] Peñalver, R., Vicedo, B., & López, M.M. (2000). Use of the genetically engineered Agrobacterium strain K1026 for biological control of crown gall. *European Journal of Plant Pathology*, 106(9), 801-810. doi: 10.1023/A:1008785813757.

[27] Qing, G.E., Huanduo, X.U., Jiale, W., Chuwei, Y.U., Wei, L., & Xu, L. (2024). Identification of pathogenic bacteria and screening of control agents for wine grape disease in the Western Sichuan Plateau. *Journal of Fruit Science*, 41(8), 1636-1648. doi: 10.13925/j.cnki.gsxb.20240116.
[28] Sawant, I. (2023). Microbes in management of fungal diseases of grape. *Grape Insight*, 1(2), 59-69. doi: 10.59904/gi.v1.i2.2023.17.
[29] Stockwell, V., Kawalek, M., Moore, L.W., Loper, J.E., Stockwell, V., Stockwell, V.O., Kawalek, M., Moore, L., Loper, J., Moore, W., & Loper, E. (1996). Transfer of pAgK84 from the biocontrol agent *Agrobacterium radiobacter* K84 to *A. tumefaciens* under field conditions. *Phytopathology*, 86, 31-37. doi: 10.1094/Phyto-86-31.
[30] Thompson, M.G., Moore, W.M., Hummel, N.F.C., Pearson, A.N., Barnum, C.R., Scheller, H.V., & Shih, P.M. (2020). *Agrobacterium tumefaciens*: A bacterium primed for synthetic biology. *BioDesign Research*, 2020, article number 8189219. doi: 10.34133/2020/8189219.
[31] Tzfira, T., Hohn, B., & Gelvin, S. (2017). Transfer of genetic information from agrobacterium to plants. *Reference Module in Life Sciences*. doi: 10.1016/B978-0-12-809633-8.07278-2.
[32] Wang, Y., Zhang, S., Huang, F., Zhou, X., Chen, Z., Peng, W., & Lu, M. (2017). VirD5 is required for efficient *Agrobacterium* infection and interacts with Arabidopsis VIP2. *New Phytologist*, 217(2), 726-738. doi: 10.1111/nph.14854.
[33] Wei, Q., Li, J.Y., Wang, J.H., & Wang, H.M. (2009). Strain E26 of *Agrobacterium vitis*, a biological control agent of grapevine , does not contain virA and virG pathogenic determinants . *Journal of Phytopathology*, 157, 657-665. doi: 10.1111/j.1439-0434.2009.01544.x.
[34] Wong, A.T., Kawaguchi, A., & Nita, M. (2021). Efficacy of a biological control agent Rhizobium vitis ARK-1 against Virginia R. *Vitis isolates*, and relative relationship among Japanese and Virginia *R. vitis* isolates. *Crop Protection*, 146, article number 105685. doi: 10.1016/j.cropro.2021.105685.
[35] Yepes, L.M., Burr, T., Reid, C., & Fuchs, M. (2019). Elimination of the pathogen, *Agrobacterium vitis*, from systemically infected grapevines bytissue culture. *American Journal of Enology and Viticulture*, 70(3), 243-248. doi: 10.5344/ajev.2019.18083.

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Бактеріальний рак виноградної лози та перспективи його біологічного контролю

Анотація. Актуальність дослідження обумовлена поширенням бактеріальних хвороб винограду на півдні України та необхідністю удосконалення методів ідентифікації патогену й засобів захисту. Метою було роботи було встановити ареал бактеріального раку винограду в Одеській області та розробити профілактичні заходи на основі біологічного методу. У роботі проводили обстеження промислових насаджень на наявність симптомів хвороби та її поширення у польових умовах. Використовували молекулярно-біологічний метод полімеразної ланцюгової реакції (ПЛР) у реальному часі для ідентифікація збудника бактеріального раку. Дослідження проводились відповідно до атестованої методики. Для ідентифікації фітопатогенів використовували обладнання ПЛР-лабораторії. У результаті фітосанітарного обстеження виноградних насаджень різних господарств в Одеській області були виявлені кущі виноградних рослин з характерними симптомами бактеріального раку, з пухлинними наростами тканини в різних місцях рослин: на рукавах, штамбі, місцях щеплення. Загалом у районі проведення досліджень бактеріальний рак винограду характеризується

широким ареалом. За результатами фітосанітарного обстеження поширення бактеріального раку винограду на різних сортах становило від 0,3 до 35 %. У режимі реального часу методом ПЛР було ідентифіковано збудника хвороби – *A. tumefaciens.* Хвороба проявлялася у вигляді характерних симптомів, а також розвивалася у латентній формі. Розроблена мультиплексна ПЛР дозволяє одразу аналізувати різні штами патогенних ізолятів агробактерій. Серед більшості виділених ізолятів, які в подальшому були протестовані на пригнічення пухлин, було виявлено два ізоляти агробактерій: ІЛВМ1 і ІЛВМ2, які мали високі показники антагоністичних властивостей щодо збудника бактеріального раку. Перевірка патогенності вилучених агробактерій на тест-рослинах томатів і соняшнику підтвердили результати визначення цих властивостей, отриманих і*n vitro*. Ізоляти агробактерій ІЛВМ1 і ІЛВМ2 значно інгібували ріст пухлин на стеблах тест-рослин порівняно з патогенним штамом, тому їх в подальшому можна використовувати проти збудника бактеріального раку і захисту виноградних рослин від вторинного ураження патогеном із ґрунту. Результати можуть бути використані для вдосконалення методів біологічного захисту рослин від бактеріальних інфекцій

Ключові слова: хвороби винограду; полімеразна ланцюгова реакція; *Agrobacterium tumefaciens*; бактерії-антагоністи; біологічний захист