Oak bark (*Quercus* sp. cortex) protects plants through the inhibition of quorum sensing mediated virulence of *Pectobacterium carotovorum*

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Abstract

Bacterial intercellular communication mediated by small diffusible molecules, known as quorum sensing (QS), is a common mechanism for regulating bacterial colonisation strategies and survival. Influence on QS by plant-derived molecules is proposed as a strategy for combating phytopathogens by modulating their virulence. This work builds upon other studies that have revealed plant-derived QS inhibitors extracted from oak bark (*Quercus* sp.). It was found that co-incubation of *Pectobacterium carotovorum* VKM-B-1247 with oak bark extract (OBE) reduced the production of acyl-HSL. This was accompanied by a dose-dependent decrease in the bacterial cellulolytic and protease activity. At the transcriptomic level, the OBE treatment suppressed the main QS-related genes *expR/expI*. Potato tubers pre-treated with OBE showed resistance to a manifestation of soft-rot symptoms. Analysis of the component composition of the OBE identified several biologically active molecules, such as n-hexadecanoic acid, 2,6-di-tert-butyl-4-methylphenol, butylated hydroxytoluene (BHT), gamma-sitosterol, lupeol, and others. Molecular docking of the binding energy between identified molecules and homology models of LuxR–LuxI type proteins allow to identify potential inhibitors. Collectively, obtained results figure out great potential of widely distributed oak-derived plant material for bacterial control during storage of potato.

Keywords Soft-rot disease · Quorum quenching · Plant-derived molecules · Plant-protection

Introduction

Plant diseases of microbial aetiology are a serious threat to human biosafety around the globe. Until recently, it was believed that bacteria cause relatively less economic damage comparing to fungi and viruses (Sundin et al. 2016). Today there is no doubt, that plant diseases caused by bacterial pathogens place major constraints on crop production and cause significant annual losses on a global scale (Sundin et al. 2016). There has been an increase in plant infections caused by *Pseudomonas syringae*, *Xanthomonas translucens*, *Pectobacterium sp.*, *Xanthomonas sp.*, *Clavibacter michiganensis*, and *Ralstonia solanacearum* (Leadbeater et al. 2014).

Good agricultural practices, cultivation and crop rotation are important tools to minimize the impact of pathogenic bacteria on crops. However, these measures do not lead to complete success, and bacterial diseases of crops are found to be difficult to control. If the list of fungicides contains hundreds of preparations, then a number of antibacterial drugs is limited to copper compounds in combination with fungicides, less often, these can be antibiotics aminoglycosides or tetracyclines used to treat fruit trees in some countries (Leadbeater et al. 2014). Antibiotic is a good solution against plant bacteriosis, but most of them are reserved for medicine practice (Sundin et al. 2016).

The phenomenon of density-dependent intercellular communication of bacteria by means of chemical molecules
known as quorum sensing (QS), was discovered at the end of the twentieth century (Fuqua et al. 1994). This finding made it possible to look from a different perspective at the functioning of microbial communities, including how the pathogenic potential of a bacterial population is realised. Moreover, it became clear that dissociation of intercellular communication seemed a promising approach to fighting infections, as long as cells remain alive, which means that the selective pressure on the population decreases, and the selection of resistant forms is unlikely (Gutiérrez-Pacheco et al. 2019; Dong et al. 2007). However, whether bacteria can become resistant to QS-inhibitors is now being questioned (Kalba et al. 2014).

The inhibition of QS is realised by various mechanisms, including enzymatic degradation, adsorption on various macromolecules, and interference with the production or perception of autoinducers (Als) (Grandclément et al. 2016). Recombinant lactonase-producing bacterial strains or transgenic lactonase-producing potato are the examples how quorum quenching could be realized in plant protection (Dong et al. 2007; Fan et al. 2020; Zhou et al. 2022).

Quorum quenchers of plant origin implement their own action through interference with the perception and biosynthesis of Als (Koh et al. 2013; Deryabin et al. 2019; Janak et al. 2021). Since numerous plant-derived molecules have structural similarities to natural Als, this allows them to compete for Al binding sites. Thus, several chemical classes of molecules related to terpenes, phenylpropanoids, flavonoids, tannins, and ellagitannins bind and disturb the functionality of LuxRI-type proteins (Deryabin et al. 2019).

Pectobacterium carotovorum is a Gram-negative bacterium that is widespread throughout the world. The ability to produce a number of plant cell wall degrading enzymes (PCWDEs) including pectinases, cellulases, and proteases (Põllumaa et al. 2012) determines the presence of this species in the list of top-10 phytopathogenic microorganisms (Mansfield et al. 2012). Pectobacterium carotovorum is responsible for causing soft-rot of potato due to the breaking the intercellular communication of P. carotovorum by the treatment with oak-derived molecules. We had found that oak bark extract has a quorum quenching activity against AHL-dependent bacterial phytopathogen. We unraveled that QQ-activity of Oak bark extract is associated with the different expression of the main QS-related genes, that on cellular level led to cupping the manifestation of P. carotovorum virulence. Using gas chromatography coupled with mass spectrometry, and molecular docking analysis, we described the molecular composition of OBE and identified the most likely inhibitors. Thus, interference of oak-derived molecules with virulence in Pectobacterium figure out great potential of widely distributed oak-derived plant material for bacterial control during storage of potato.

Material and methods

Bacterial strains

Pectobacterium carotovorum (Jones) Waldee VKM B-1247 was obtained from the All-Russian Collection of Microorganisms (Pushchino, Russia). This strain produces 3-oxohexanoyl-L-homoserine lactone (3-oxo-C6-HSL) as it was revealed by high performance liquid chromatography coupled with mass spectrometry (HPLC–MS) (Figure S5).

C. violaceum CV 026 is a mini-Tn5 mutant of wild type ATCC 31532, deficient in QS dependent violacein production was used as a biosensor for detecting exogenous acyl-HSL. C. violaceum CV026 produces purple pigment in response to short chain acyl-HSL.

Biomolecular sensors strain of Escherichia coli MG 1655 pVFR1-lux is a bioluminescence-based QS-biosensor (Manukhov et al. 2011). This strain carries the plasmid pVFR1, which possesses the luxRI’::luxCDABE (meaning luxI mutated) bioluminescent reporter gene. This system can detect acetylated homoserine lactones (acyl-HSL) with acyl chains ranging from six to twelve carbons in length (C6 to C12 acyl-HSLs).

Extract preparation

Pharmacological preparation of chopped oak bark (Registration Number N001007/01) produced by pharmacological company “Farmatsvet” (Russia) has been used in this work. Oak bark of Quercus sp. was collected in Krasnodar region of Russia in August 2020.
Extract from oak bark was prepared by extraction with an organic solvent. A weighted portion of 10 g of chopped oak bark was poured into 100 mL of 70% ethanol (v/v), and stirred for an hour at 25 °C. The resulting suspension was centrifuged at 9000 rpm for 15 min, and then supernatant was filtered through PVDF-membrane with 0.22 µm pore diameter (VWR, US). The resulting solution was frozen in liquid nitrogen and lyophilized.

**Gas chromatography–mass spectrometry of oak bark extract**

Analysis of oak bark extract was performed as described in (Deryabin and Tolmacheva 2015). Gas chromatography-mass spectrometry (GC–MS) study was carried out on a Trace GC Ultra chromatograph with a DSQ II mass-selective detector in the electron ionization mode (70 eV). Thermo TR-5 MS quartz capillary column 15 m long, 0.25 mm inner diameter, with a stationary phase film thickness of 0.25 µm was used. Split-free input mode was used. Helium gas was used as the carrier gas at a constant flow rate of 1 mL/min. Evaporator temperature 250 °C, junction chamber temperature 250 °C. The temperature of the column thermostat was changed according to the program: from 80 (delay 5 min) to 290 °C at a rate of 15 °C/min. The total analysis time is 29 min. The volume of the injected sample is 10.0 µl. Mass scanning range 40–500 amu.

**Growth curves**

*Pectobacterium carotovorum* VKM-B-1247 was grown overnight at 28 °C in 4 mL LB medium (tryptone—10 g/L, yeast extract—10 g/L, NaCl—2 g/L, glucose—0.5%) under continuous shaking at 150 rpm. Prepared bacteria for 106 CFU/mL subsequently have been inoculated to 96-well microtiter plates (Eppendorf, US) containing growth media and various concentrations of oak bark extract in series of two-fold dilutions. The dynamics of bacterial growth were assessed by reading and plotting the absorbance data at 620 nm obtained by the spectrophotometer Multiscan GO (Thermo Scientific, US).

**Qualitative determination of acyl-HSL formed by* P. carotovorum* under the influence of oak bark extract**

The overnight grown *P. carotovorum* culture was seeded on soft agarose LB-medium (0.75% v/v) supplemented with oak bark extract (0.5 and 1.0 mg/mL). *P. carotovorum* was checked for acyl-HSL production by striking against biosensor strain *C. violaeceum* CV026 on soft agarose medium. The plates were incubated at 28 °C for 24 h and AI-dependent pigment production in CV026 was visually observed. The purple pigment production in CV026 was indicative of diffusible acyl-HSL produced by *P. carotovorum*.

**Quantification of acyl-HSL formed by* P. carotovorum* under the influence of oak bark extract**

*Pectobacterium carotovorum* was cultured in a volume of 10 mL in LB medium (tryptone—10, yeast extract—10, glucose—0.5%, NaCl—2 g/L) at 28 C, 95 rpm. Upon reaching the stage of the exponential growth, 100 µl of oak bark extract dilutions were added to the cells up to the final appropriate concentrations. The cells were subsequently cultured.

At hourly intervals, 1.5 mL of each cell suspension was collected. Bacteria were precipitated by centrifugation at 10 000 rpm/15 min, 6 °C. The supernatant was transferred into a separate tube for the quantitative analysis of autoinducers using the bioluminescence assay. Bacterial biomass was frozen in liquid nitrogen and stored at − 80 °C for subsequent isolation of nucleic acids.

**Bioluminescence assay**

**Quantitative detection acyl-HSL using biosensor *E. coli* MG1655 pVFR1-lux**

Bioluminescence assay was performed as described in work (Julian et al. 2021). Supernatants of *P. carotovorum* cultures were mixed with biosensor *E. coli* MG 1655 pVFR1-lux in 96-well microtiter plates to a final volume of 100 µL. Bioluminescence was recorded as relative light units (RLU) using Multiscan FL reader (Thermo, USA) every 5 min at 25 °C. The resulting values of bioluminescence were estimated in induction factor (R), which was processed according to the following Eq. 1:

\[
R = \frac{I_{t_{120} \text{ min}} - I_{t_{0} \text{ min}}}{I_{c_{120} \text{ min}} - I_{c_{0} \text{ min}}}
\]

where \( I_t \) is intensity of bioluminescence of a treated sample; \( I_c \) is intensity of bioluminescence of the control sample (in the absence of inducer).

**RNA sample collection and gene expression analysis using reverse transcription and quantitative Real-Time PCR (RT-qPCR)**

**RNA extraction and cDNA preparation**

Total RNA was isolated using the Quick-RNA Microprep kit (Zymo Research) according to the manufacturer’s instruction. RNA was quantified using Qubit RNA HS Assay Kit (Thermo Fisher Scientific, US). Total RNA was further treated with DNase I (New England Biolabs) followed by the RNA Clean & Concentrator-5 kit (Zymo Research). The
absence of contaminating DNA was verified by PCR. Then, using the reverse transcription reaction, complementary DNA was obtained using the iScript reverse transcription supermix for RT-qPCR reagent (Bio-Rad, US).

Quantification of mRNA by quantitative real-time PCR (qRT-PCR)

Quantitative PCR was carried out with the obtained cDNA using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, USA). Each reaction mix with a volume of 20 µl was prepared with 300 nM each primer (final concentration) and 20 ng of cDNA. Light Cycler96 Real-Time PCR detection system (Roche, Switzerland) was used for the measurements using a protocol with the following thermal cycling conditions: DNA denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 10 s and annealing/extension at 60 °C for 15 s. After the last amplification cycle, a melting curve analysis was carried out by heating from 65 to 95 °C in increments of 0.5 °C/s. Negative controls (without template or reverse transcriptase enzyme) were included in each run. Gene-specific primers (described in Table S1) for the genes used in RT-qPCR experiments were designed using online service Integrated DNA technologies (https://www.idtdna.com/Primerquest/Home/Index). Fold changes in the expression levels of the investigated genes were normalized in relation to the levels of the housekeeping genes rho and recA mRNA.

Effect of oak bark extract on production of plant cell wall degrading enzymes (PCWDEs)

Pectinolytic activity of P. carotovorum VKM-B-1247 was assessed as described in work (Roy et al. 2018). Briefly, P. carotovorum was grown on medium containing yeast extract 1%, pepticin 1%, agar 1.5%, and NaCl 0.5% (pH 7.0) at 37 °C for 48 h. Then, clear zones upon flooding with iodine-potassium iodide solution (1.0 g iodine, 5.0 g potassium iodide, and 330 mL H2O) were considered as bacterial pectinase activity.

Cellulase and protease degrading potential of P. carotovorum was qualitatively estimated by calculating hydrolysis capacity, that is the ratio of diameter of halo zone and colony.

Effect of oak extract on virulence of P. carotovorum by a potato maceration assay

Assay based on method described in Singh et al. with our modifications (Singh et al. 2021). Potato tubers were purchased from the market and washed under tap water. The potato slices (0.5 cm) were surface sterilized by treating with 3% hydrogen peroxide for 30 min followed by washing with sterile distilled water. Each slice was placed in a sterile Petri dishes containing wet filter paper. Prepared water suspension of the oak bark extract (1 mg/mL) or sterile water (control group) were scattered to the surface of potato slices and dried in the air. Bacterial suspension of 106 CFU/mL was dropped at the center of each slice. The Petri dishes were incubated at 28 °C for 76 h.

In silico analysis

Homology modeling of CviR/CviI of C. violaceum.

Homology model of acyl-homoserine lactone synthase CviI of C. violaceum is based on its UniProt sequence (Q83XU6) and crystal structure of the homologous protein TofI from Burkholderia glumae deposited in protein data bank (PDB ID: 3P2H), which has endogenous ligand 5'-deoxy-5'-methylthioadenosine resolved in the catalytic site (Chung et al. 2011) and shares 26% of identity with the target. These values are above the 20% threshold considered to be appropriate for homology modeling (Joshi et al. 2016).

The 3D homology model of CviI was built using Prime in Schrödinger Suite (Schrödinger, Inc., US), sequences were aligned with Prime STA. Protein Reliability Report tool was utilized to assess binding pocket stability revealing no deviations from acceptable values.

CviR is a transcriptional regulator of C. violaceum which structure was downloaded from the RCSB website (PDB ID: 3QP5) in the form of crystal structure bound to antagonist chloroacetone (Chen et al. 2011). The protein structures were prepared using the Protein Preparation Wizard implemented in Maestro.

Docking

The ligand structures were downloaded from the PubChem website. LigPrep tool was utilized to prepare ligands using the OPLS3 force field generating low energy ionized and tautomeristic states at pH 7.0 ± 2.0, while chirality was determined from ligands 3D structure. Receptor grid box was centered using co-crystallized ligands and extended to 12 Å. Bound ligands were extracted and then was re-docked along
with a set of optimized ligands of interest using Glide XP. Docking scores are demonstrated in the results section.

**Statistical analysis**

The experiments were performed using two independent series with 3 to 10 technical replicates each. The obtained results were statistically analyzed with Origin 2018 (Origin-Lab Corporation, Northampton, Massachusetts, USA) software.

The Shapiro–Wilk test was used to assess the normal distribution of values. In the presence of a normal distribution, the Student’s t-test has been used. The data are presented as mean and standard deviation (mean ± SD). Differences were considered significant at p-value ≤ 0.05.

**Results**

**Antibacterial effect of oak bark extract, and its influence on biosynthesis of acyl-homoserine lactone**

First, the oak bark extract (OBE) was evaluated for its antimicrobial properties. We were unable to detect the inhibition of *P. carotovorum* cells growth by OBE taken at concentrations of 0.25–1.0 mg/mL (Figure S1).

In the second step, we assessed the OBE quorum quenching activity. The growth of *P. carotovorum* is accompanied by the diffusion of C6-oxo-HSL AIs, which are perceived by the sensory strain *Chromobacterium violaceum* CV026 (Fig. 1). It has been shown that Pectobacteria produces less AIs when growing on a medium containing OBE (Fig. 1b–d) compared to a medium without any supplements (Fig. 1a).

Thus, a qualitative suppression of the production of *P. carotovorum* AIs by OBE was established. The quantitative effect of the addition of OBE on AI production was assessed using the bioluminescent strain of *E. coli* pVFR1-lux. The induction of bioluminescence is proportional to the inducer concentration in the medium. It was found that OBE inhibited the biosynthesis of C6-oxo-HSL in a dose-dependent manner. The inhibition effect was most pronounced when adding OBE at a concentration of 1 mg/mL, this concentration reduced AIs biosynthesis by 86% (Fig. 2). At the same time, no suppression of the viability of bacterial cells was observed (Figure S2).

**Expression of quorum sensing-regulated genes in the presence of oak bark extract**

The effect of the extract at the molecular level was assessed using quantitative PCR, targeting the main regulatory genes of the QS system. It was found that the expression of *expI* and *expR* genes was affected under the influence of OBE. The most significant dysregulation was shown for the *expI* gene (Fig. 3a). Co-incubation of *P. carotovorum* with 0.5 mg/mL of OBE for 2 h did not lead to a decrease in *expI* expression compared to the mock-inoculated controls, but 1.0 mg/mL was able to reduce gene expression. During the
third hour of treatment, \( \text{expI} \) expression was significantly decreased at all tested concentrations, compared with the mock-inoculated controls. In contrast, the transcription level of the \( \text{expR} \) gene in the mock-inoculated controls had no changes over time, but under the treatment (0.5 and 1.0 mg/mL) were decreased (Fig. 3b).

**Effect of oak bark extract on the production of cell wall degrading enzymes**

The used strain of \( P. \text{carotovorum} \) VKM-B-1247 was tested for its ability to produce various enzymes involved in the degradation of plant tissues. The used strain demonstrated protease and cellulase activity, but did not show pectinase activity. At the same time, the inhibition of protease and cellulase activity was found at all concentrations of OBE (Fig. 4). The virulence factor that was the most affected by the treatment was the protease activity. Thus, the addition of 0.5 mg/mL of OBE reduced the protease activity of pectobacterium by 50%, while 1.0 mg/mL of OBE reduced the protease activity of pectobacterium by more than 90%. At the same time, the cellulase activity was reduced by 60% in response to 1.0 mg/mL OBE in comparison with the mock-inoculated controls.

**Effect of oak bark extract on \( P. \text{carotovorum} \) virulence**

It is expected that a decrease in the biosynthesis of C6-oxo-HSL leads to a reduction in PCWDE activity and, in turn, to a decrease in the manifestations of the disease caused by \( P. \text{carotovorum} \). To assess this, we infected the potato tuber with a suspension of \( P. \text{carotovorum} \).

It turned out that the pre-treatment of potato tubers with OBE slowed down the development of symptoms of tissue maceration, compared with the control group. So, on the
first day on the untreated potato slices signs of maceration appeared (Fig. 5a). After 72 h, the mass of diseased tissue was about 20% of the mass of the whole potato (Fig. 5g). In turn, the blackening of the tissues of potatoes treated with OBE was not observed even by the third day of incubation (Figs. 5d–f), and the percentage of affected tissue remained less than 10% (Fig. 5g).

**GC–MS analysis of oak bark extract**

Gas chromatographic analysis of the oak bark extract allowed us to obtain mass spectra for 11 different substances with varying degrees of homology according to the NIST reference database (Table 1, Figure S3).

Among the identified components, the main part was re-presented by lipophilic polyphenolic substance 2,2′-methylenebis(6-tert-butyl-4-methylphenol) (relative content 21.3%), n-hexadecanoic acid (8.6%), plant phytosteroid gamma-sitosterol (4.3%), terpenoid friedelan-3-one (3.4%), phenolic antioxidant 2,6-di-tert-butyl-4-methylphenol (3.3%), (5), pentacyclic triterpenoid lupeol (3.2%), 1-(5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-2-ol (1.76%) and (9β)-9,19-Cyclolanostan-3β-ol acetate (1.2%). Other identified components were presented in insignificant amounts (< 1%).

**Molecular docking analysis**

The mechanism determining the suppression of QS-dependent bacterial virulence must be sought in the intermolecular interaction of small molecules with the main components of the QS system. There are two crucial components in QS system: acylated homoserine lactone (acyl-HSL) producing enzymes and LuxR-type signal receptors (Chen et al. 2011). We used molecular docking to assess the binding capacity of the identified molecules (Table 2) to the active sites of these proteins.

Molecular docking of various ligands with LuxR–LuxI proteins requires their resolved crystal structures. However, only the structure of LuxR-type protein CviR is presented in Protein Data Bank (PDB ID: 3QP5). Considering the ability of oak bark extract to inhibit QS-dependent violacein biosynthesis in *C. violaceum* ATCC 31532 (Figure S4), we

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**Table 1** The phytochemicals identified in the *Quercus cortex* extract by gas chromatography–mass spectrometry

| No | Component                                      | Relative content, % |
|----|-----------------------------------------------|---------------------|
| 1  | 1-(5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-2-ol | 1.76                |
| 2  | 2,6-di-tert-butyl-4-methylphenol               | 3.25                |
| 3  | n-Hexadecanoic acid                           | 8.6                 |
| 4  | 2,2′-Methylenebis(6-tert-butyl-4-methylphenol) | 21.3                |
| 5  | Squalene                                      | 1.61                |
| 6  | Stigmasterol acetate                          | 0.98                |
| 7  | Gamma-sitosterol                              | 4.34                |
| 8  | Lupeol                                        | 3.17                |
| 9  | (9β)-9,19-Cyclolanostan-3β-ol acetate         | 1.2                 |
| 10 | Friedelan-3-one                               | 3.43                |

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**Fig. 5** Inhibition of *P. carotovorum* virulence on potatoes. Potato discs coated with oak bark extract (1 mg/mL) before bacteria were inoculated (10⁶ CFU/mL). Representative photos of development of soft-rot symptoms on untreated (a–c) and pretreated with OBE (d–f). Photos were made at 24 (a, d), 48 (b, e), and 72 h (c, f). Plant-protection effect was determined as the percentage of macerated tissue 72 h after the inoculation of potato tubers with *P. carotovorum* (g). Treatments not connected by the same letter in each panel are significantly different from each other (p < 0.05; means ± SD).
performed in silico analysis using available structure CviR and generated homology model CviI.

CviI acyl-homoserine-lactone synthase homology model was built on the crystal structure of the homologous protein TofI (PDB ID: 3P2H) analogous to the previously described approach Jiang et al. (2013). Using homology modelling, we calculated the active site composition of \textit{C. violaceum} CviI acyl-homoserine-lactone synthase to provide a model for testing acyl-HSL producing enzyme inhibitors. Gamma-sitosterol demonstrated a slightly lower docking score (– 6.888 kcal/mol) compared to the reaction by-product 5′-deoxy-5′-methylthioadenosine (–7.553 kcal/mol), which was resolved in the TofI crystal structure and re-docked to the CviI homology model (Fig. 6a, b). Other candidates for potential acyl-HSL producing enzyme inhibitors were (9β)-9,19-Cyclolanostan-3β-ol acetate (–6.364 kcal/mol), 1-(5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-2-ol (–6.296 kcal/mol), 2,2′-Methylenebis(4-methyl-6-tert-butylphenol) (–5.835 kcal/mol), and Butylated hydroxytoluene (–7.397 kcal/mol) (Table 2).

To our predictive model for the CviR transcriptional regulator, 2,2′-Methylenebis(4-methyl-6-tert-butylphenol) (Fig. 6d) and 1-(5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-2-ol have slightly higher binding energy (–7.876 and –7.739 kcal/mol, respectively) than the known inhibitor chlorolactone (7.222 kcal/mol) (Fig. 6c) and thus can be analysed as potential QS signal blockers. 2,2′-Methylenebis(4-methyl-6-tert-butylphenol) and 1-(5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-2-ol bind several amino acids that play role in the chlorolactone interaction with the protein (Trp84, Tyr88, Asp97, Ser155) (Fig. 6d), therefore, a similar mechanism can be proposed. Docking analysis revealed that several compounds in OBE can possibly affect the functioning of both acyl-HSL producing enzymes and LuxR-type signal receptors.

Discussion

This work is a continuation of study series aimed to discover and apply structure-functional analysis of plant-derived substances with quorum quenching activity. The bark and

| Table 2 Docking results of complexes from CviI and CviR proteins | CviR/Ligand | Docking score (kcal mol\(^{-1}\)) |
|---|---|---|
| 2,2′-Methylenebis(4-methyl-6-tert-butylphenol) | | – 7.876 |
| 1-(5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-2-ol | | – 7.739 |
| (9β)-9,19-Cyclolanostan-3β-ol acetate | | – 5.634 kcal/mol |
| Butylated hydroxytoluene | | – 7.397 |
| Chlorolactone | | – 7.222 |
| gamma-Sitosterol | | – 6.555 |
| [5-(Hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl]methanol | | – 5.675 |
| Hexadecanoic acid | | – 5.485 |
| Squalene | | – 4.751 |
| Friedelan-3-one | | – 3.221 |
| CviI/ligand | | |
| 5′-Deoxy-5′-methylthioadenosine | | – 7.553 |
| (resolved in the crystal structure, re-docked) | | |
| Gamma-Sitosterol | | – 6.888 |
| (9β)-9,19-Cyclolanostan-3β-ol acetate | | – 6.364 |
| 1-(5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-2-ol | | – 6.296 |
| 2,2′-Methylenebis(4-methyl-6-tert-butylphenol) | | – 5.835 |
| Lupeol | | – 4.947 |
| Butylated hydroxytoluene | | – 4.438 |
| Squalene | | – 2.538 |
| Stigmasterol acetate | | – 2.468 |
| Friedelan-3-one | | – 2.438 |

Target: \textit{C. violaceum} CviR transcriptional regulator (PDB ID: 3QP5); and \textit{C. violaceum} CviI acyl-homoserine-lactone synthase homology model built on TofI from \textit{Burkholderia glumae} structure (PDB ID: 3P2H).
leaves of some oaks (*Quercus* spp.) are recognised to be pharmacological agents used in various countries for the treatment and prevention of infectious processes caused by viruses, bacteria, fungi, and other pathogens (Assessment report EMA/HMPC/3206/2009).

Regarding bacteria, the oak bark extract (OBE) is well known as a substance with bactericidal and bacteriostatic properties, while its quorum quenching activity has been discovered recently (Adonizio et al. 2006). Existing works have revealed details on molecular composition of OBE which determines its quorum quenching properties (Deryabin and Tolmacheva 2015). However, the precise mechanism at the core of OBE quorum quenching properties is still unraveled, as well as how OBE could be used to reduce microbial virulence.

We estimated what extract of oak bark can potentially be considered natural active substance to prevent plant disease caused by *P. carotovorum*. Interestingly, we found that the direct antimicrobial effect of OBE against *C. violaceum* and *P. carotovorum* is absent. In contrast, the anti-quorum activity of the OBE was pronounced on *C. violaceum*, as well as *P. carotovorum*. This is not surprising, given that the QS system of *P. carotovorum* is based on the LuxI-LuxR regulatory system.

At the next stage, we carried out a quantitative assessment of the ability of oak bark extract to suppress the functioning of quorum sensing of pectobacteria. It turned out that co-incubation of the test strain with the studied drug inhibited the biosynthesis of QS-autoinducers. The implementation of suppression starts at the first hour after adding of OBE and reaches its maximum at the third hour of exposure.

The mechanism of blocking quorum sensing was investigated at the transcriptomic and phenotypic levels. It turned out that oak bark extract reduces the expression of *expR* and *expI*, the products of which are proteins of reception and synthesis of acyl-HSL, respectively.

Thus, oak bark extract affects the expression of QS-related genes, that leads to reducing biosynthesis of AIs and subsequently affects enzymatic activity of cellulases and proteases. In the ecology of pectobacteria, the processes of plant tissues colonization are under the control of the QS system. After getting attached to the surface of plants, pectobacteria produce various enzymes that destroy plant tissues. Therefore, the production of cellulases, pectinases and glycosyl hydrolases allows plant pathogenic bacteria to destroy the cell wall firstly, which provides the bacterial population with carbohydrates and allows it to penetrate inside plant tissues (Gorshkov 2017). In turn, proteases of phytopathogens destroy lectins, deactivate PR-proteins, mitogen-activated protein kinase (MAPK) and activate plant immunity and extensins that strengthen the structural integrity of the plant cell wall (Dow et al. 1998; Tendiuk et al. 2022; Savidor et al. 2012; Ek-Ramos et al. 2019; Gur-Arie et al. 2020).

Analysing the molecular composition of oak bark extract, we used the method of gas chromatography with mass spectrometric detection. This method has proven to be useful...
itself well in the study of the chemical composition of various plant extracts (Husain et al. 2017) including oak bark extract (Deryabin and Tolmacheva 2015). We found that none of the substances we identified had previously been found in the composition of the oak bark extract. The oak bark extract obtained in the work of Deryabin and Tolmacheva (2015) contained at least five different QS inhibitors, including vanillin, and coumarin derivatives. These molecules were not found in our samples. Two main reasons led to such results: first of all, the origin of biological material and the composition of the bark which admittedly belongs to different Quercus spp. (for instance, Q. robur and Q. pubescens); secondly, in this work we made soft trial preparation excepting high pressure and temperature during autoclaving that may lead to partial chemical degradation of components located inside the bark.

Nevertheless, a number of identified molecules can potentially act as inhibitors of quorum sensing. It was previously reported that n-hexadecanoic acid (palmitic acid) demonstrated the ability to inhibit bacterial biofilm development and reduce quorum sensing mediated gene expression in Vibrio harveyi (Santhakumari et al. 2017). The antioxidants 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene) (Jiang et al. 2013) and 2,2′-Methylenebis (6-tert-butyl-4-methylphenol) (Jang et al. 2017) are known for their antiviral and anticarcinogenic properties. Lupeol is a pharmacologically active pentacyclic triterpenoid (Margareth et al. 2009). It has anti-cancer and anti-inflammatory activity and slightly antimicrobial activities. It is important to note that lupeol has been shown to inhibit the violacein production of C. violaceum (Bodede et al. 2018). The triterpenoid friedelan-3-one has been reported to exhibit antioxidant and anti-inflammatory activities (Sunil et al. 2013) derived from the leaf of Pterocarpus santalinoides showed antibacterial activity (Ichiko et al. 2016). Then docked in CviR, the higher scores were calculated for 2,2′-methylenebis(4-methyl-6-tert-butyphenol) and 1-((5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-y)]propan-2-ol. It is better cores than for the known antagonist chloroalocate. Interaction modeling with CVil demonstrated the best docking scores for gamma-Sitosterol and 2,2′-Methylenebis(4-methyl-6-tert-butyphenol) compared to other tested compounds.

**Conclusion**

Thus, we determined that oak bark water–ethanol extract is the preparation that quite effectively inhibits quorum sensing mediated virulence of P. carotovorum and causes potato spoilage. Molecular modelling has shown that a number of oak-derived molecules is able to be incorporated into the active centre of both the I-protein and the R-protein, which could determine quorum quenching properties of Oak bark extract. However, it is unlikely that quorum quenching property of oak bark extract is related to each one molecule in the mixture. More likely, the suppression of bacterial intercellular communication is due to the additive and diverse effects of a complex of molecules constituting the plant-derived extract.

It’s important to notice, that reproducing the oak bark extract composition which retains the QQ-property is a first step to developing a novel biological control strategy against AHL-dependent bacterial phytopathogens.

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**Author contributions** ASV conceived and designed the study. DVP, AVI, RS, AVV, EAR performed the experiments. ASV, DVP, RS, AVV, EAR analyzed and interpreted the data. ASV, EAR drafted and wrote the manuscript. ASV supervised the study. All authors approved the final version of the manuscript.

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**Declarations**

**Conflict of interest** No conflict of interest declared.

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