Antibacterial and Anti-Virulence Effects of Furazolidone on *Trueperella Pyogenes* and *Pseudomonas Aeruginosa*

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Abstract

Background: *Trueperella pyogenes* and *Pseudomonas aeruginosa* are two important bacterial pathogens closely relating to the occurrence and development of forest musk deer respiratory purulent disease. Although *T. pyogenes* is the causative agent of the disease, the subsequently invaded *P. aeruginosa* will predominate the infection by producing a substantial amount of quorum-sensing (QS)-controlled virulence factors, and co-infection of them usually creates serious difficulties for veterinary treatment. In order to find a potential drug that targets both *T. pyogenes* and *P. aeruginosa*, the antibacterial and anti-virulence capacities of 55 compounds, which have similar core structure to the signal molecules of *P. aeruginosa* QS system, were tested in this study. By performing a series of *in vitro* screening experiments to assess the effects of these compounds.

Results: We identified that furazolidone could significantly inhibit the growth of mono-cultured *T. pyogenes* or in the co-culture with *P. aeruginosa*. Although the growth of *P. aeruginosa* could also be moderately inhibited by furazolidone, the results of phenotypic identification and transcriptomic analysis further revealed that furazolidone had remarkable inhibitory effect on the biofilm production, motility, and QS system of *P. aeruginosa*. Moreover, furazolidone could efficiently protect *Caenorhabditis elegans* from *P. aeruginosa* infection under both fast-killing and slow-killing conditions.

Conclusions: This study reports the antibacterial and anti-virulence abilities of furazolidone on *T. pyogenes* and *P. aeruginosa*, and provides a promising strategy and molecular basis for the development of novel anti-infectious drugs to dealing with forest musk deer purulent disease, or other diseases caused by *T. pyogenes* and *P. aeruginosa* co-infection.

Background

Forest musk deer (*Moschus berezovskii*) is an important economic animal and is endemic to China. The musk secreted by male forest musk deer is a precious Chinese medicine and important raw material of high-grade spice. Purulent disease usually manifests as purulent lesions on the epidermis, uterus, and internal organs, and is one of the main reasons hindering the growth of forest musk deer population [1, 2]. Compared to the body surface abscesses which can be easily observed and removed artificially, the internal purulent lesions are extremely harmful due to the complex bacterial composition, and also are difficult to be detected in time [1, 3]. Therefore, internal abscesses are usually found dying deer and have brought huge challenge and economic loss to forest musk deer breeding industry.

Our prior work has shown that *Trueperella pyogenes* and *Pseudomonas aeruginosa* are two main bacterial pathogens in the respiratory suppurative lesion of forest musk deer, with *T. pyogenes* as the primary pathogen and *P. aeruginosa* as the dominate species in the lateral stage [1, 2, 4]. *T. pyogenes* is Gram-positive, pleomorphic, non-spore forming, inactive, non-enveloped and facultative anaerobic belonging to the family *Actinomycetaceae*, and a resident bacterium of the skin and mucous membranes of the animal respiratory tract, digestive tract, and genitourinary tract [5, 6, 7, 8]. This microorganism can reduce the oxygen content and oxidation-reduction potential of the infection site by producing the key

virulence factor pyolysin through fermentation metabolism, and this process is beneficial to other bacterial species [9, 10].

P. aeruginosa is a ubiquitous opportunistic Gram-negative bacterium that can infect a variety of host tissues and cause acute and chronic infections [11, 12]. It is well-recognized that the quorum-sensing (QS) system, which is activated by specific signal molecules (acyl-homoserine lactones, AHLs) and coordinates the production of virulence factors and intracellular metabolism of many bacterial species, plays an important role in the processes of bacterial invasion and cell-cell communications [3, 13, 14, 15]. The QS system of *P. aeruginosa* is composed of three hierarchically arranged regulatory networks. The *las*- and *rhl*-QS systems have complete signal molecule synthesis proteins (LasI/RhlI) and regulatory proteins (LasR/RhlR), and the activation of *rhl* is mainly depended on *las*. The *pqs* system only has the regulatory protein *pqsR*, and the activation of which requires *Pseudomonas* quinolone signal from other pathways co-regulated by *lasR* and *rhlR* [14].

It is considered that inhibiting the virulence of pathogenic bacteria by targeting the QS system is a promising strategy for the development of novel anti-infectious drugs, namely QS inhibitors or anti-virulence drugs [16]. We have previously shown that the natural AHL signals of *P. aeruginosa* QS system could inhibit the growth and virulence of *T. pyogenes* [17]. *P. aeruginosa* has strong competitive advantage over *T. pyogenes* when any of the three QS regulators of *P. aeruginosa* was knocked out [18]. Therefore, we hypothesize that there might be a kind of drug can simultaneously inhibit the growth or virulence of *T. pyogenes* and *P. aeruginosa*. Hence, in this study, we tested the antibacterial and anti-QS activities of 55 small molecule drugs with similar core structure to that of *P. aeruginosa* QS signals. Finally, we identified that furazolidone could inhibit the growth of *T. pyogenes* and *P. aeruginosa* and showed a strong inhibitory activity on the QS system of *P. aeruginosa*.

Results

Screening of compounds inhibit the growth of T. pyogenes

For the 55 compounds with similar core structure to the AHL signals of *P. aeruginosa* QS system, we first tested the inhibitory effects of them on the growth of *T. pyogenes*. We found that although the majority of the compounds had no effect on the growth of *T. pyogenes* TP13 in BHI-FBS broth compared with the control, 9 of them were found to have significant growth inhibition effect and 8 have significant growth enhancement effect (Additional file 1 Supplementary Table S1). Nitrofurantoin, nitrofurazone, ronidazole, and furazolidone, which could significantly inhibit *T. pyogenes* TP13 in a dose-dependent manner (Fig. 1), were selected to treat co-cultured *T. pyogenes* and *P. aeruginosa*.

Furazolidone inhibits the growth of T. pyogenes and P. aeruginosa

T. pyogenes TP13 and *P. aeruginosa* PAO1 were well-mixed into different ratios (1:1, 1:9 and 9:1) and co-cultured in BHI-FBS agar supplemented with 200 μM of nitrofurantoin, ronidazole, furazolidone, and nitrofurazone. In agreement with our prior finding that *P. aeruginosa* had an innate growth advantage in

the competition with *T. pyogenes* [17], *P. aeruginosa* would always be the dominant species under the co-culture condition with *T. pyogenes*, irrespective of their initial ratios (Fig. 2). The addition of the compounds, especially nitrofurazone, significantly suppressed the growth of *T. pyogenes* TP13 but had different effects on the growth of *P. aeruginosa* PAO1 in the co-culture. Notably, among of the 4 tested compounds, only furazolidone could simultaneously inhibit the growth of *T. pyogenes* TP13 and *P. aeruginosa* PAO1 compared to the control (Fig. 2).

Furazolidone inhibits the QS-related phenotypes of P. aeruginosa

We found that furazolidone showed a dose-dependent growth inhibition effect on *P. aeruginosa* PAO1, but would not totally clean them (Additional file 1 Supplementary Fig. S1). Considering the similar core structure of furazolidone to the AHL signals of *P. aeruginosa* QS system, we then tested the possibility that furazolidone might have a negative effect on the QS regulation of *P. aeruginosa* PAO1. Because the rapid population proliferation of *P. aeruginosa* using adenosine or skim milk as the sole carbon source requires the intracellular hydrolase or extracellular protease elastase that are positively regulated by the QS system, and thus the growth status of *P. aeruginosa* under these conditions can be used to preliminarily evaluate the performance of QS system [19]. As shown in Table 1, furazolidone significantly suppressed the growth of *P. aeruginosa* PAO1 on M9-adenosine plates, and had a dose-dependent inhibition effect on the production of extracellular proteases on M9-skim milk plates. Moreover, we further showed that the production of biofilm and pyocyanin and the swimming and twitching motilities of *P. aeruginosa* PAO1 could also be significantly inhibited by furazolidone (Fig. 3).

Table 1
Inhibitory effect of furazolidone on the growth of *P. aeruginosa* PAO1 on M9-adenosine and M9-skim milk plates.

Furazolidone (μM)	M9-adenosine[a]	M9-skim milk (cm)[b]
0	+	1.72 ± 0.061
50	–	1.57 ± 0.050****
100	–	1.41 ± 0.074****
200	–	1.32 ± 0.050****

[a] "+", Normal growth. "–", Inhibited growth.

[b] Data shown are the mean ± standard deviation of nine independent experiments. One-way ANOVA, **** $p < 0.0001$.

Furazolidone inhibits the QS-regulation of P. aeruginosa

To further investigate the effect of furazolidone on the QS regulation of *P. aeruginosa*, RNA-sequencing was then used to profile the global transcription change of furazolidone-treated *P. aeruginosa* PAO1. The results showed that compared to the control, 465 up-regulated genes and 107 down-regulated genes were

identified in *P. aeruginosa* PAO1 cultured in LB broth supplemented with 200 μM of furazolidone (Fig. 4A and Additional file 2). Prediction of KEGG pathway revealed that the functions of flagellar assembly, bacterial chemotaxis, propanoate metabolism, ribosome, and degradation of valine, leucine and isoleucine were significantly enriched among the up-regulated genes ($p < 0.05$), while QS system was the sole significantly enriched KEGG term among the down-regulated genes (Fig. 4B).

We then explored the suppression effect of furazolidone on *P. aeruginosa* QS system in more detail by applying all the significantly changed genes to the list of QS-induced genes previously released by Schuster [20]. We found that among the 315 QS-induced genes, 63 of them including the typical genes relating to the common QS-activated phenotypes, were screened from the 107 down-regulated genes (Fig. 4C). The result of quantitative PCR further confirmed that compared to the control, the expression levels of three key regulatory genes (*lasR*, *rhlR*, and *pqsR*) and their downstream functional genes (*lasB, rhlA, pqsA, pqsD, pqsE*, *hcnA*, and *phzA*) were all down-regulated by 1.5–8.2 folds in furazolidone-treated *P. aeruginosa* (Fig. 4D). Moreover, we also checked the effect of furazolidone on the expression levels of the main virulence factors of *T. pyogenes* TP13 and found that, the supplementation of furazolidone significantly decreased the expression of *plo*, *ploS*, *ploR*, *cbpA*, *fimA*, *nanH*, and *nanP* by 1.7–10.7 folds in *T. pyogenes* TP13 compared to that of untreated group (Additional file 1 Supplementary Fig. S2).

Furazolidone protects C. elegans **from** P. aeruginosa **infection**

We then tested the *in vivo* protection activity of furazolidone against *P. aeruginos*a infection by using *C. elegans* as a model. In the fast-killing assay which mimics the acute infection condition, all the *C. elegans* were killed by *P. aeruginosa* PAO1 in 80 hours in the untreated group (Fig. 5A), while furazolidone treatment significantly increased the survival rate of *C. elegans* ($p < 0.0001$). In the slow-killing assay which mimics the chronic infection condition, *P. aeruginosa* PAO1 killed all the *C. elegans* in 8 days in the absence of furazolidone, while the death of *C. elegans* in furazolidone-treated group was slowed down in the initial 5 days and stopped on day 7 (Fig. 5B). Finally, the survival curve of *C. elegans* in furazolidone-treated group was significantly different from that of untreated group ($p = 0.0102$). Therefore, these results suggested that furazolidone could protect *C. elegans* from *P. aeruginosa* PAO1 challenge.

Discussion

Forest musk deer is a solitary ruminant vulnerable to various diseases and hurts from the environments, and among which the respiratory suppurative lesion is a major disease threatening the healthy growth of forest musk deer [1, 2]. The present study identifies that furazolidone can simultaneously inhibit the growth and virulence of *T. pyogenes* and *P. aeruginosa*, which are the two important bacterial pathogens closely related to the occurrence and development of the respiratory suppurative disease of forest musk deer [2, 4, 17].

Previous studies have reported that the QS signals of *P. aeruginosa* can inhibit the growth and virulence of *T. pyogenes in vitro* and *in vivo* [17, 21], and thus provides an explanation for the replacement of dominant bacteria from *T. pyogenes* to *P. aeruginosa* during the development of the respiratory

suppurative disease of forest musk deer. This can also be supported by our current data that *P. aeruginosa* is ultimately the dominant strain in the mixed co-culture with *T. pyogenes*, irrespective of their initial ratios (Fig. 2). In comparison to the Gram-positive bacterium *T. pyogenes* which is normally isolated from animals and shows relatively less extent of antibiotic resistance, *P. aeruginosa* is a notorious Gram-negative bacterium with a variety of innate or acquired abilities to resist the clearance of antibiotics [22]. Moreover, reducing bacterial virulence by antagonizing QS system has been suggested to be an evolutionarily robust anti-infectious strategy [16, 23]. These findings further hint that there might be a kind of drugs which is lethal to *T. pyogenes* but can inhibit the QS system of *P. aeruginosa*.

It is reported that the commonly used clinical antibiotics azithromycin, ceftazidime, and ciprofloxacin could function as QS inhibitors of *P. aeruginosa* to improve the clinical outcome of patients [24, 25, 26]. Some natural products such as the extract of *Dalbergia Trichocarpa* bark, baicalin (an active natural compound extracted from the traditional Chinese medicinal *Scutellaria baicalensis*), and sodium ascorbate could also inhibit the QS-regulated virulence of *P. aeruginosa* [27, 28, 29]. According to the principle deduced above, we first tested the antibacterial activities of 55 compounds with similar core structure to the AHL signals of *P. aeruginosa* QS system on mono-cultured *T. pyogenes* and co-cultured *T. pyogenes* and *P. aeruginosa*. Furazolidone, which could profoundly inhibit the growth of *T. pyogenes* TP13 and slightly inhibit *P. aeruginosa* PAO1, was finally screened (Figs. 1 and 2). Furazolidone is a nitrofuran antibiotic that can be used to treat gastrointestinal diseases such as dysentery, enteritis, and gastric ulcer caused by bacteria and protozoa [30]. In the present study, we further discovered the anti-virulence activity of furazolidone by showing that, the presence of furazolidone significantly reduced the production of QS-regulated virulence factors, biofilm formation, and cell motilities of *P. aeruginosa* PAO1 (Table 1 and Fig. 3). As determined by the transcriptomic analysis, furazolidone decreased the expression of 107 genes of *P. aeruginosa* PAO1, and 63 of them were positively regulated by QS (Fig. 4A, C). The result of qPCR further demonstrated that furazolidone could inhibit the expression of all the three central regulatory genes (*lasR*, *rhlR*, and *pqsR*) and their downstream functional genes (*lasB*, *rhlA, pqsA*, *pqsD*, *pqsE*, *hcnA*, and *phzA*) of *P. aeruginosa* PAO1 (Fig. 4D). These data indicated that QS system was the major target inhibited by furazolidone in *P. aeruginosa* PAO1.

On the other hand, the presence of furazolidone up-regulated the expression of 465 genes and significantly promoted the flagellar assembly, chemotaxis, and several metabolic pathways of *P. aeruginosa* PAO1 (Fig. 4B). Although it is not clear whether these enhanced abilities would contribute to the fitness of *P. aeruginosa* in the host tissue, the result of *C. elegans* killing experiments demonstrated that furazolidone was capable of efficiently protecting *C. elegans* from *P. aeruginosa* PAO1 infection, especially in the fast-killing assay (Fig. 5). We failed to measure the protection ability of furazolidone on *C. elegans* against *T. pyogenes* challenge. This is because the virulence of *T. pyogenes* is relatively moderate compared to the common bacterial pathogens and not sufficient to kill *C. elegans* even when the cell density was up to $1.0 \subseteq 10^9$ CFUs/ml (Data not shown). However, we found that furazolidone could inhibit the expression of the known virulence factor-encoding genes (Additional file 1 Supplementary Fig. S2).

Conclusion

Collectively, our data here suggested that furazolidone could be considered as an antibacterial and anti-virulence drug simultaneously inhibit the growth and virulence of *T. pyogenes* and *P. aeruginosa*. Further animal model-based mechanistic study combined with structure optimization of furazolidone would contribute to the development of novel drugs to treat the respiratory suppurative disease of forest musk deer.

Methods

Bacterial strains and media

T. pyogenes TP13 isolated from the lung pus of forest musk deer [4] and wild-type (WT) *P. aeruginosa* strain PAO1 were preserved in the lab and used elsewhere [15]. All the strains were routinely cultured in brain heart infusion with 5% fetal bovine serum (BHI-FBS) or in lysogeny broth (LB) from a single colony.

Culture conditions

A total of 55 compounds (Additional file 1 Supplementary Table S1) with similar core structure to the Acyl-homoserine lactones (AHL) signals of *P. aeruginosa* QS system were selected and purchased from MedChemExpress (Shanghai, China). Overnight cultured *T. pyogenes* TP13 was diluted to optical density of 1.0 at wavelength of 600 nm (OD_{600}=1.0) by sterile saline solution. Equal amount of *T. pyogenes* (10 μL) was inoculated in 200 μL of BHI-FBS medium containing different concentrations (0, 50, 100, and 200 μM) of compounds and culture at 37°C overnight, and then the cell densities were determined at OD_{600}. Subsequently, *T. pyogenes* TP13 and *P. aeruginosa* PAO1 were mixed (1:9, 1:1, and 9:1) and co-cultured overnight on BHI-FBS agar containing 200 μM of the compounds with significant growth inhibition activities on *T. pyogenes*. The composition of *T. pyogenes* and *P. aeruginosa* in the co-culture were determined by counting the colony forming units (CFUs) of them on BHI-FBS agar plates after appropriate dilution, because the phenotypes of *T. pyogenes* and *P. aeruginosa* are significantly different and can be easily discriminated. Finally, the compounds that could inhibit the growth of *T. pyogenes* and *P. aeruginosa* were added (0, 50, 100, and 200 μM) to 200 μL of LB medium containing 10 μL of *P. aeruginosa* and cultured overnight at 37°C. The cell densities were determined at OD_{600}. All the experiments above were independently repeated for three times.

Quorum-sensing inhibition assay

M9-adenisine (0.1%, wt/v) and M9-skimmed milk (0.5%, wt/v) agar medium were used to evaluate the inhibitory activity of compounds on *P. aeruginosa* QS regulation [19]. Overnight cultured *P. aeruginosa* PAO1 was adjusted to OD_{600}=1.0 and inoculated (5 μL) on M9-adenisine agar and M9-milk agar containing different concentrations (0, 50, 100, and 200 μM) of compounds. The growth status of *P. aeruginosa* on M9-adenisine plates and the diameter of proteolytic circle formed on M9-milk plates were determined after 24 hours. The experiments were independently repeated for nine times.

Biofilm production assay

Equal amount (20 μl, OD_{600}=1.0) of *P. aeruginosa* PAO1 was inoculated in glass tubes containing 2 mL of LB broth supplemented with different contractions (0, 50, 100, and 200 μM) of compounds, and overnight cultured at 37°C with shaking (220 rpm). The cell density was measured at OD_{600}. After the culture solution and unadhered biofilm were gently removed, the adhered biofilm on the tube wall was stained with crystal violet (0.1%) for 30 minutes and washed with PBS buffer for three times. Subsequently, the stained biofilm was dissolved by 95% of ethanol solution and quantified at OD_{595}. The experiments were independently repeated for three times.

Pyocyanin production assay

Equal amount (20 μl, OD_{600}=1.0) of *P. aeruginosa* PAO1 was inoculated in glass tubes containing 2 mL of LB broth supplemented with different contractions (0, 50, 100, and 200 μM) of compounds, and overnight cultured at 37°C with shaking (220 rpm). After the cell density was equalized with fresh LB broth, 200 μL of bacterial solution was taken out to extract the pyocyanin by chloroform and 0.2 N HCl and measured at OD_{520} as described by Essar [31]. The experiments were independently repeated for three times.

Motility assay

For the swimming motility assay, 5 μl (OD_{600}=1.0) of *P. aeruginosa* PAO1 was inoculated on the surface of LB plates containing 0.5% of agar supplemented with different contractions (0, 50, 100, and 200 μM) of compounds and cultured at 37°C for 24 h. For the twitching motility assay, 2 μl (OD_{600}=1.0) of *P. aeruginosa* PAO1 was stabbed into the bottom of LB plates containing 1.0% of agar supplemented with different contractions (0, 50, 100, and 200 μM) of compounds and cultured at 37°C for 24 h. The motilities of *P. aeruginosa* PAO1 were determined by measure the diameters of colony on the surface (swimming motility) or the thin film region on the bottom (twitching motility). All the experiments were independently repeated for six times.

Transcriptomic analysis

Bacterial cells of furazolidone-treated and -untreated *P. aeruginosa* PAO1 were harvested for total RNA isolation using TRIzol reagents (Invitrogen), respectively. RNAs samples were conducted for library construction and RNA-sequencing (RNA-seq) by Novogene Bioinformatics Technology Company using prokaryotic strand-specific Illumina-based RNA-Seq technology (HiseqTM2500 platform). The obtained clean reads were mapped to the reference genome of PAO1 (NCBI accession number: AE004091) by the software Tophat2 [32]. SOAP2 program [33] and Cufflinks [34] were used to calculate the expected fragments per kilobase of transcript per million fragments (FPKM) sequenced, and the differentially expressed transcripts were presented and analyzed by EdgeR [35]. Differentially expressed gene with false discovery rate $p < 0.05$ was thought to be significantly different. The significantly differently expressed genes were mapped to the list of QS-activated genes reported by Schuster [20] by using VENNY 2.1 (http:// bioinfogp.cnb.csic.es/tools/venny/).

Quantitative PCR

Total RNAs of furazolidone-treated and -untreated *P. aeruginosa* PAO1 were isolated by using TRIzol reagents, and the cDNA was synthesized by reverse transcription using a high-capacity cDNA Reverse Transcriptase kit Specific with gDNA removal (Takara). Quantitative PCR was performed by using an iTaq™ universal SYBR® Green Supermix (Bio-Rad) and the CFX Connect Real-Time PCR Detection System to validate the expression of typical QS-activated genes including *lasR*, *rhlR*, *pqsR*, *lasB*, *rhlA*, *pqsA*, *pqsD*, *pqsE*, *hcnA*, and *phzA* (Additional file 1 Supplementary Table S2). Gene expression was calculated by the $2^{-\Delta\Delta CT}$ method using 16S rRNA as reference.

Caenorhabditis elegans assay

For the fast-killing assay, 20 µl (OD_{600}=1.0) of *P. aeruginosa* PAO1 was spread on peptone-glucose-sorbitol (PGS) agar media with and without furazolidone, and cultured overnight at 37°C. The naturally cooled plates were seeded with 10 newly cultured adult *C. elegans* (L4 stage) and further incubated at 25°C for 96 hours. For the slow-killing assay, to prevent *C. elegans* from laying eggs, 40 µL of 5-fluoro-2'-deoxyuridine solution (40 µg/mL) was evenly coated on the surface of nematode growth medium (NGM). Subsequently, 20 µl (OD_{600}=1.0) of *P. aeruginosa* PAO1 was spread on NGM plates with and without small molecule drugs and cultured overnight at 37°C. The naturally cooled plates were seeded with 10 newly cultured adult *C. elegans* and further incubated at 25°C for 10 days. The survival status of *C. elegans* in each experiment were observed and recorded. Growth of *C. elegans* on PGS agar plates or NGM plates feed with uracil auxotrophy *Escherichia coli* OP50 were set as controls.

Statistical analyses

Data analysis and statistical tests were performed by using Graphpad Prism version 9.0 (San Diego, CA, USA). Mean values of standard deviation were compared by using two-tailed unpaired t-test or One-way ANOVA. The survival curves of *C. elegans* were compared by using Log- rank (Mantel-Cox) test.

Abbreviations

AHL: Acyl-homoserine lactones

BHI: brain heart infusion

CFUs: colony forming units

FBS: fetal bovine serum

FPKM: fragments per kilobase of transcript per million fragments

LB: lysogeny broth

NGM: nematode growth medium

PCR: Polymerase chain reaction

PGS: peptone-glucose-sorbitol

QS: quorum-sensing

WT: wild-type

Declarations

Ethics approval and consent to participate

not applicable

Consent for publication

not applicable

Availability of data and materials

The data shown in this paper are available within the article and supplementary materials. The raw data of RNA- sequencing are available from the NCBI database under accession number PRJNA723215 (SRR14368535 and SRR14368537) https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA723215.

Competing interests

The authors declare no conflict of interest exists.

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Authors' contributions

Experiment and data processing: Q. CHEN. K. L. ZHAO. H. Y. LI. K. H. LIU. J. LI. Wrote the manuscript: Q. CHEN. Revised the manuscript: K. L. ZHAO. Y.W.CHU. B. Prithiviraj. B. S. YUE. X. Y. ZHANG. The author(s) read and approved the final manuscript.

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Figures

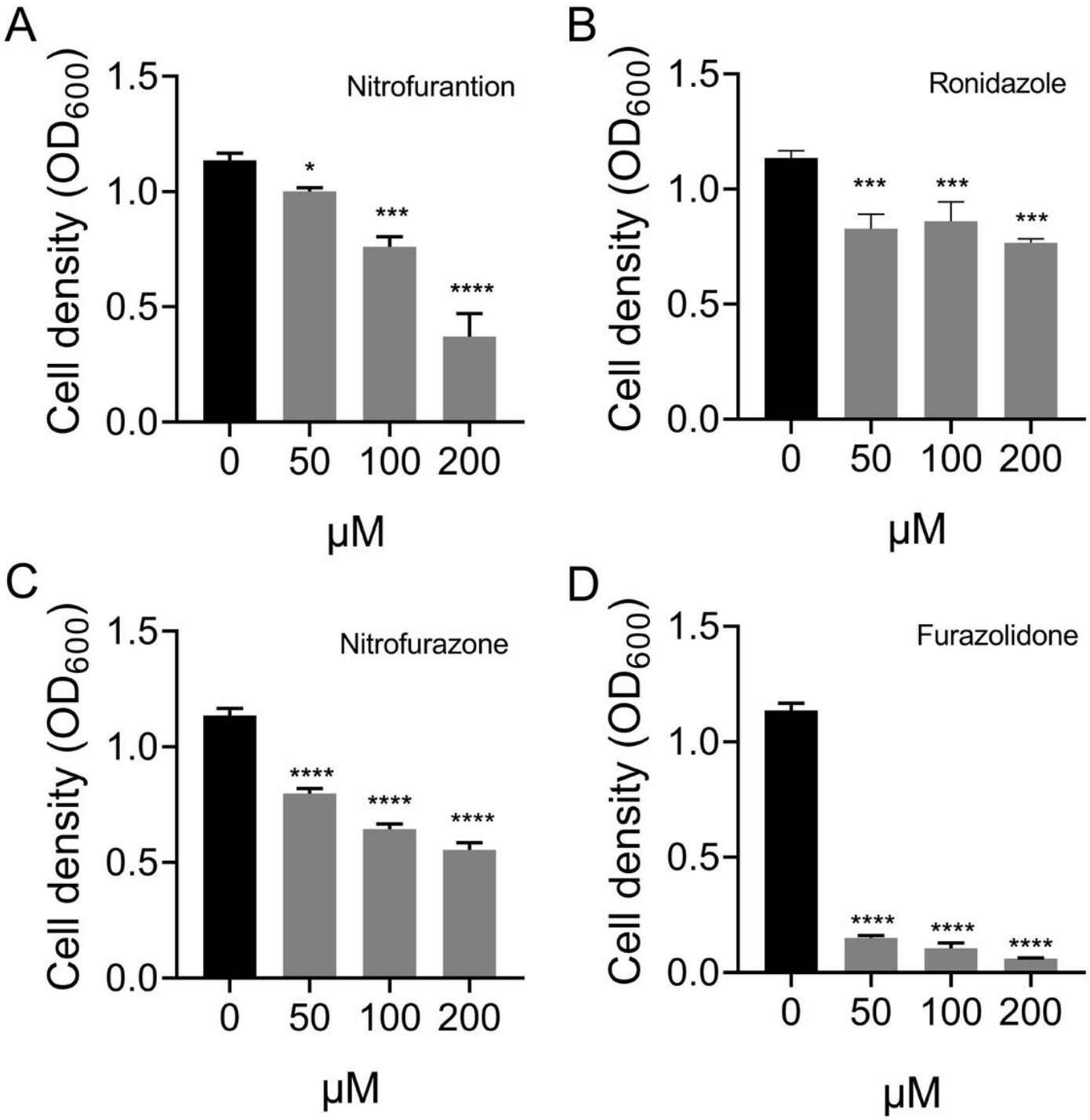

Figure 1

Effects of different compounds on the growth of Trueperella pyogenes TP13. Equal amount of T. pyogenes TP13 was inoculated in brain heart infusion (BHI) broth containing 5% fetal bovine serum (FBS) and different concentrations of (A) nitrofurantoin, (B) ronidazole, (C) nitrofurazone, or (D) furazolidone, and cultured overnight at 37°C. Data shown are the mean ± standard deviation (SD) of three independent experiments. One-way ANOVA, * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

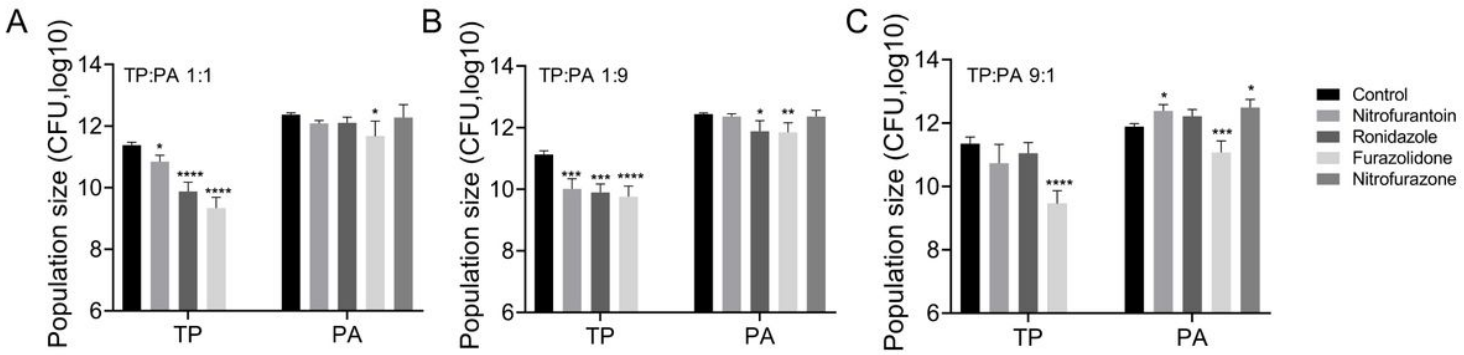

Figure 2

Effects of different compounds on the growth of T. pyogenes TP13 and Pseudomonas aeruginosa PAO1 under different co-culture conditions. T. pyogenes TP13 and P. aeruginosa PAO1 were mixed and co-cultured on BHI+5% FBS plates containing 200 μM of nitrofurantoin, ronidazole, nitrofurazone, or furazolidone from the initial ratios of (A) 1:1, (B) 1:9, and (C) 9:1, and cultured overnight at 37°C. Data shown are the mean ± SD of three independent experiments. One-way ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

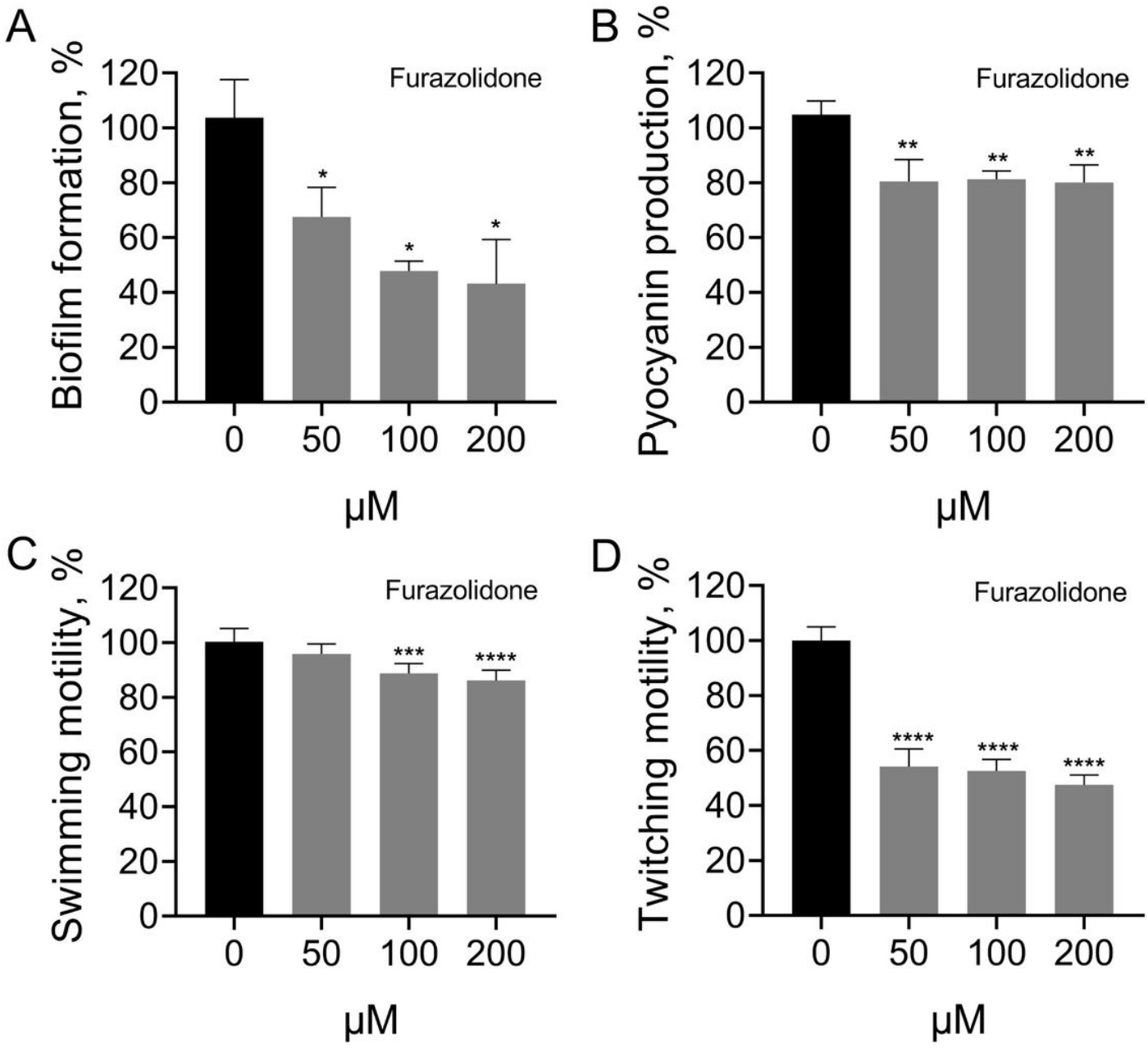

Figure 3

Inhibitory effect of furazolidone on the (A) biofilm formation, (B) pyocyanin production, (C) swimming motility and (D) twitching motility of P. aeruginosa PAO1. Data shown are the mean ± SD of three independent experiments. One-way ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

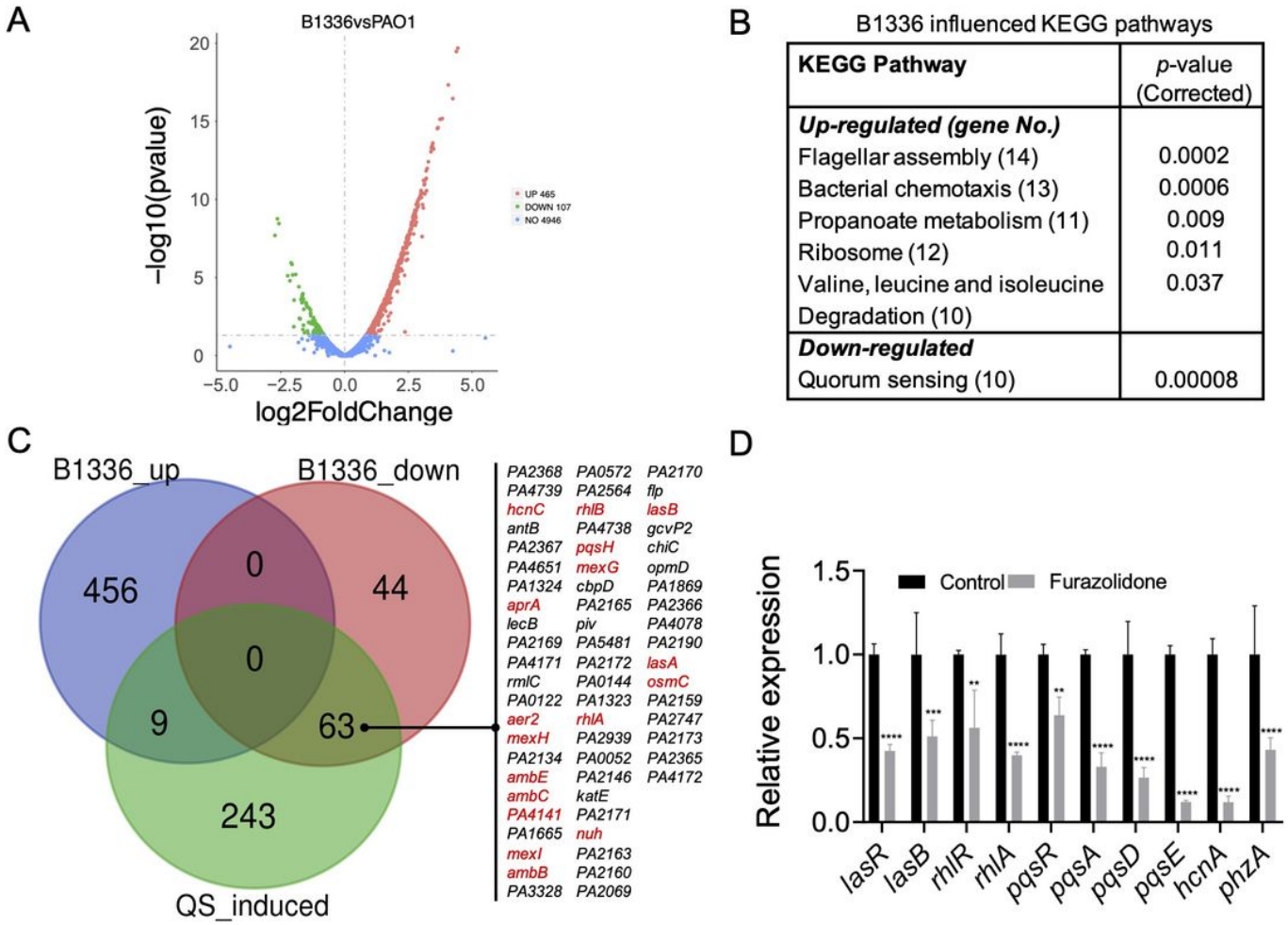

Figure 4

Effects of furazolidone on the global transcription of P. aeruginosa PAO1. (A) Volcano plotting of differentially expressed genes. B1336 indicates furazolidone treated PAO1. (B) Significantly influenced KEGG pathways of P. aeruginosa PAO1 by furazolidone. (C) The significantly differentially expressed genes of P. aeruginosa PAO1 were applied to the list of QS-induced genes published by Schuster et al. (2003). (D) Expression of typical QS-regulated genes of furazolidone-treated PAO1 as determined by qPCR. Data shown are the mean ± SD of three independent experiments. One-way ANOVA, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

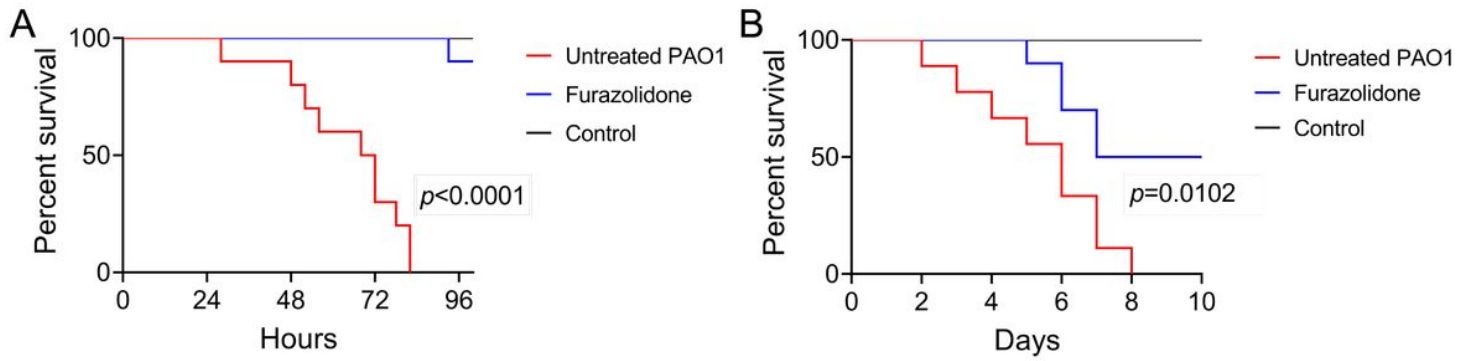

Figure 5

Pathogenicity of P. aeruginosa PAO1 can be reduced by furazolidone. Survival of Caenorhabditis elegans models challenged by P. aeruginosa PAO1 under (A) fast-killing and (B) slow-killing conditions (10 nematodes per group). Data shown are representative of six independent replicates. The survival curves were compared by using Log-rank (Mantel-Cox) test.

Supplementary Files

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