Potential Use of Dimethyl Sulfoxide in Treatment of Infections Caused by *Pseudomonas aeruginosa*

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Dimethyl sulfoxide (DMSO) is commonly used as a solvent to dissolve water-insoluble drugs or other test samples in both *in vivo* and *in vitro* experiments. It was observed during our experiment that DMSO at noninhibitory concentrations could significantly inhibit pyocyanin production in the human pathogen *Pseudomonas aeruginosa*. Pyocyanin is an important pathogenic factor whose production is controlled by a cell density-dependent quorum-sensing (QS) system. Investigation of the effect of DMSO on QS showed that DMSO has significant QS antagonistic activities and concentrations of DMSO in the micromolar range attenuated a battery of QS-controlled virulence factors, including rhamnolipid, elastase, and LasA protease production and biofilm formation. Further study indicated that DMSO inhibition of biofilm formation and pyocyanin production was attained by reducing the level of production of an autoinducer molecule of the *rhl* QS system, *N*-butanoyl-L-homoserine lactone (C₄-HSL). In a mouse model of a burn wound infection with *P. aeruginosa*, treatment with DMSO significantly decreased mouse mortality compared with that for mice in the control group. The capacity of DMSO to attenuate the pathogenicity of *P. aeruginosa* points to the potential use of DMSO as an antipathogenic agent for the treatment of *P. aeruginosa* infection. As a commonly used solvent, however, DMSO's impact on bacterial virulence calls for cautionary attention in its usage in biological, medicinal, and clinical studies.

Pseudomonas aeruginosa is a prevalent opportunistic pathogen capable of causing various infections in humans, including pneumonia and urinary tract infections, bloodstream infections, and infections in burn patients (1). The chronic infection caused by *P. aeruginosa* and the associated pulmonary inflammation are ultimately responsible for the majority of cases of mortality in patients with cystic fibrosis (2). The ability of *P. aeruginosa* to cause diverse infections is attributed to its myriad virulence factors and biofilm-forming capability, which are controlled by the intercellular quorum-sensing (QS) communication system (3–5).

P. aeruginosa has two acyl-homoserine lactone (AHL)-mediated QS systems, known as the *las* and *rhl* QS systems. The *las* and *rhl* systems consist of the transcriptional activators LasR and RhlR, respectively, and the signal synthases LasI and RhlI, respectively. The major signals in the *las* and *rhl* systems are *N*-(3-oxododecanoyl)-HSL (3-oxo-C₁₂-HSL) and *N*-butanoyl-L-homoserine lactone (C₄-HSL), respectively (5). *P. aeruginosa* employs these QS systems to control a wide range of extracellular virulence factors, including pyocyanin, elastase, and rhamnolipid (7–12). The AHL-mediated QS systems also play a crucial role in biofilm formation by *P. aeruginosa*, a common cause of resistance to antibiotics and the difficulties with the treatment of infections (13). The *las* system influences the activation of *pel* and, accordingly, biofilm matrix formation (14), and the *rhl* system contributes to the maintenance of the biofilm architecture through the production of rhamnolipid surfactants (15, 16). Due to the crucial role of QS systems in regulating virulence and biofilm formation, inhibition of the QS systems provides an alternative approach for treating *P. aeruginosa* infections. Extensive studies have focused on searching for drugs capable of blocking QS and attenuating pathogenicity.

Dimethyl sulfoxide (DMSO) is an important polar aprotic solvent that is frequently used as a vehicle in both *in vivo* and *in vitro* experiments, including in quorum sensing inhibitor (QSI) screen-

ing studies (17–20). It was unexpectedly observed during our experiments that DMSO could significantly inhibit the production of the pigmented compound pyocyanin, an important pathogenic factor in *P. aeruginosa* that is tightly regulated by the QS systems. Besides its cryoprotective and tissue penetration-enhancing actions, DMSO has been used to treat numerous conditions and ailments in preclinical research and, in some cases, clinical situations. The conditions treated include dermatologic diseases (21), pain (22), chronic prostatitis (23), gastrointestinal disorders (24–27), wound healing (6), pulmonary fibrosis and amyloidosis (28, 29), and interstitial cystitis (IC) (30), even though in most cases the pharmacological mechanisms are unknown.

In this study, we investigated the effect of DMSO on the pathogenicity of *P. aeruginosa*. We present data showing that DMSO exhibits significant antagonistic activities on QS-associated virulence factors in *P. aeruginosa*, such as rhamnolipid, elastase, and LasA protease production and biofilm formation. The mechanism of DMSO attenuation of virulence factors was found to be the reduction of the level of production of C₄-HSL, the signal molecule of the

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TABLE 1 Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
Strains		
<i>E. coli</i>		
SM10-λpir	Mobilizing strain, RP4 integrated in the chromosome, Kn ^r	Invitrogen
DH10B	F [−] <i>mcrA</i> Δ(<i>mrr-hsdRMS-mcrBC</i>) ϕ80 <i>dlacZ</i> Δ <i>M15</i> Δ <i>lacX74</i> <i>deoR recA1 endA1 araD139</i> Δ(<i>ara leu</i>)7697 <i>galU galK</i> λ [−] <i>rpsL nupG</i>	Invitrogen
<i>P. aeruginosa</i>		
PAO1	Wild type	55
PDO100	<i>rhlI</i> mutant of PAO1	
Plasmids		
pMS402	Expression reporter plasmid carrying the promoterless <i>luxCDABE</i> gene, Kn ^r Tmp ^r	31
CTX6.1	Integration plasmid origin of plasmid mini-CTX- <i>lux</i> , Tc ^r	This study
pKD- <i>phzA1</i>	pMS402 containing <i>phzA1</i> promoter region, Kn ^r Tmp ^r	31
pKD- <i>phzA2</i>	pMS402 containing <i>phzA2</i> promoter region, Kn ^r Tmp ^r	31
pKD- <i>lasI</i>	pMS402 containing <i>lasI</i> promoter region, Kn ^r Tmp ^r	41
pKD- <i>rhlI</i>	pMS402 containing <i>rhlI</i> promoter region, Kn ^r Tmp ^r	41
pKD- <i>rhlA</i>	pMS402 containing <i>rhlA</i> promoter region, Kn ^r Tmp ^r	31
pKD- <i>gacA</i>	pMS402 containing <i>gacA</i> promoter region, Kn ^r Tmp ^r	31
pKD- <i>rpoS</i>	pMS402 containing <i>rpoS</i> promoter region, Kn ^r Tmp ^r	31
pKD- <i>pilG</i>	pMS402 containing <i>pilG</i> promoter region, Kn ^r Tmp ^r	31
pKD- <i>fliC</i>	pMS402 containing <i>fliC</i> promoter region, Kn ^r Tmp ^r	31
pKD- <i>pqsA</i>	pMS402 containing <i>pqsA</i> promoter region, Kn ^r Tmp ^r	65
pKD- <i>pqsR</i>	pMS402 containing <i>pqsR</i> promoter region, Kn ^r Tmp ^r	65
pKD- <i>pqsH</i>	pMS402 containing <i>pqsH</i> promoter region, Kn ^r Tmp ^r	32
pKD- <i>exoS</i>	pMS402 containing <i>exoS</i> promoter region, Kn ^r Tmp ^r	31
pKD- <i>exoY</i>	pMS402 containing <i>exoY</i> promoter region, Kn ^r Tmp ^r	31
pKD- <i>exoT</i>	pMS402 containing <i>exoT</i> promoter region, Kn ^r Tmp ^r	31
pKD- <i>exsD</i>	pMS402 containing <i>exsD</i> promoter region, Kn ^r Tmp ^r	31
pKD- <i>exsC</i>	pMS402 containing <i>exsC</i> promoter region, Kn ^r Tmp ^r	31
CTX- <i>exoS</i>	Integration plasmid CTX6.1 with a fragment of pKD- <i>exoS</i> containing the <i>exoS</i> promoter region and <i>luxCDABE</i> gene, Kn ^r Tmp ^r Tc ^r	This study
CTX- <i>exoY</i>	Integration plasmid CTX6.1 with a fragment of pKD- <i>exoY</i> containing the <i>exoY</i> promoter region and <i>luxCDABE</i> gene, Kn ^r Tmp ^r Tc ^r	This study
CTX- <i>exoT</i>	Integration plasmid CTX6.1 with a fragment of pKD- <i>exoT</i> containing the <i>exoT</i> promoter region and <i>luxCDABE</i> gene, Kn ^r Tmp ^r Tc ^r	This study
CTX - <i>phzA1</i>	Integration plasmid CTX6.1 with a fragment of pKD- <i>phzA1</i> containing the <i>phzA1</i> promoter region and <i>luxCDABE</i> gene, Kn ^r Tmp ^r Tc ^r	This study
CTX - <i>phzA2</i>	Integration plasmid CTX6.1 with a fragment of pKD- <i>phzA2</i> containing the <i>phzA2</i> promoter region and <i>luxCDABE</i> gene, Kn ^r Tmp ^r Tc ^r	This study

rhl system. The use of DMSO for the treatment *P. aeruginosa* infection was examined using a mouse model of burn wound infection.

MATERIALS AND METHODS

Materials, plasmids, and culture conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *P. aeruginosa* PAO1 and its derivatives were routinely grown at 37°C on Luria-Bertani (LB) agar plates or in LB broth with orbital shaking at 200 rpm. Where appropriate, antibiotics were used at the following concentrations: for *E. coli*, kanamycin (Kn) was used at 50 μg/ml, and for *P. aeruginosa*, tetracycline (Tc) was used at 300 μg/ml in *Pseudomonas* isolation agar (PIA) and trimethoprim (Tmp) was used at 300 μg/ml in LB broth. DMSO was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as indicated below.

Monitoring of gene expression. The plasmid pMS402 containing a promoterless *luxCDABE* reporter gene cluster was used to construct promoter-reporter fusions of virulence-related genes as described previously (31). Virulence gene expression in liquid cultures was monitored on the basis of light production from the promoterless *luxCDABE* operon downstream of the gene promoter (32). Overnight cultures of the reporter strains were diluted to an optical density at 600 nm (OD₆₀₀) of 0.2 and cultivated for an additional 2 h before use as inoculants. The cultures were

inoculated into parallel wells in a 96-well black plate with a transparent bottom. Aliquots of a fresh culture (5 μl) were inoculated into the wells, which contained 95 μl of medium with or without DMSO (2%, vol/vol). To prevent evaporation during the assay, 50 μl of filter-sterilized mineral oil (Sigma) was added. Both luminescence and bacterial growth (OD₆₀₀) were measured every 30 min for 24 h in a Victor³ multilabel counter (PerkinElmer, USA). The level of light production, measured in counts per second (cps), was proportional to the level of gene expression. The light production values were then normalized to the level of bacterial growth. The level of gene expression is presented as the number of relative luminescence units (RLU), calculated as cps/OD₆₀₀ by use of the normalized cps values.

To monitor gene expression on solid medium, the reporter strains were plated onto soft-top LB agar. Overnight cultures of the reporter strains were diluted to an OD₆₀₀ of 0.2. The upper layer of medium (LB medium with 0.7% agar), cooled to 40°C, was mixed with 100 μl of the diluted cultures supplemented with DMSO (2%, vol/vol) or 100 μl of the diluted cultures alone (as control), and the mixture was spread onto the lower layer of medium (LB medium with 1% agar). The plates were incubated overnight in 37°C, and imaging was performed using a LAS3000 imaging system (Fuji Corp.).

Measurement of pyocyanin production. Pyocyanin was extracted from the supernatants of overnight cultures as described previously (33) with minor modifications. Briefly, PAO1 cultures were grown in LB medium supplemented with DMSO (2%, vol/vol) or LB medium not supplemented with DMSO (as a control). Three milliliters of chloroform was added to 5 ml of the culture supernatant. After extraction, the chloroform layer was transferred to a fresh tube and mixed with 1 ml of 0.2 M HCl. After centrifugation, the top layer (0.2 M HCl) was removed, and the absorption at 520 nm was measured. The concentrations obtained, expressed as micrograms of pyocyanin produced per milliliter of culture supernatant, were calculated using an extinction coefficient of 17.072 at 520 nm (34).

Elastase quantitation. The production of elastase in *P. aeruginosa* cultures was determined by the elastin-Congo red assay with minor modifications (35). PAO1 cultures were grown in LB medium in the presence or in the absence (as a control) of DMSO (2%, vol/vol) to an OD₆₀₀ of 2.0. The cells were harvested by centrifugation, and the supernatants were filter sterilized (syringe filters from Techno Plastic Products, Trasadingen, Switzerland; pore size, 0.22 µm). The filter-sterilized supernatants or LB medium was mixed 2:1 with phosphate buffer (0.1 M, pH 6.3), and 2 mg/ml elastin-Congo red (Sigma) was then added. The mixture was incubated at 37°C with shaking (200 rpm) for 3 h. After centrifugation, the absorbance of the supernatant at 495 nm was measured in a spectrophotometer. The elastin-Congo red sample incubated with medium alone was used as the blank. The experiment was performed in triplicate with supernatants from three independent experiments.

LasA staphylolytic activity assay. LasA protease activity was determined by measuring the ability of culture supernatants to lyse boiled *Staphylococcus aureus* cells (36). Briefly, an overnight culture of *S. aureus* cells was boiled for 10 min and centrifuged for 10 min at 10,000 × g. The resulting pellet was resuspended in 10 mM Na₂PO₄ (pH 7.5) to an OD₆₀₀ of approximately 0.8. A 100-µl aliquot of supernatant of a *P. aeruginosa* overnight culture treated or not treated with DMSO was added to 900 µl of a boiled *S. aureus* suspension. Medium alone and medium with DMSO were used as the blank controls, respectively. The OD₆₀₀ was determined after 0, 5, 10, 20, 30, 45, and 60 min.

Rhamnolipid detection. Rhamnolipid was detected in *P. aeruginosa* culture fluids as described previously (37). Cells from mid-exponential-phase cultures grown in peptone tryptone soya broth (5% peptone, 0.25% tryptone soya broth, pH 7.0) were washed and resuspended in modified GS medium (Medium Group C) (38) supplemented or not supplemented with DMSO when they reached an OD₆₀₀ of 0.2 and then incubated at 37°C with shaking. The cultures were incubated for a total of 80 h and then centrifuged at 16,000 × g for 5 min. The supernatants were sterilized by passing them through 0.22-µm-pore-size filters, and the filtrates were extracted twice with 2 volumes of diethyl ether. The pooled ether extracts were then extracted once with 20 mM HCl, and the ether phase was evaporated to dryness. Finally, the residue was dissolved in water, and the rhamnolipid content in each sample was determined by comparison with rhamnose standards via duplicate orcinol assays (39), where 1.0 mg rhamnose corresponded to 2.5 mg rhamnolipid.

Quantification of biofilm formation. Biofilm formation was measured in a static system as described previously (40) with minor modifications. Cells from overnight cultures were standardized to an OD₆₀₀ of 0.2 and inoculated at a 1:10 dilution. The fresh cultures in 96-well polystyrene microtiter plates (Costar) were supplemented with DMSO (2%, vol/vol) or were not supplemented with DMSO as a control. After 24 h of incubation at 37°C without agitation, crystal violet solution (1%, wt/vol) was added to each sample well at a volume equal to one-fourth of the culture volume, and the mixture was incubated at room temperature for 15 min. The wells were washed twice with distilled water and filled with 200 µl of 95% ethanol to solubilize the crystal violet. A 125-µl aliquot of this solution was transferred to a new polystyrene microtiter plate, and the absorbance at 570 nm was measured. All experiments were independently

repeated 3 times, and 8 replicate samples were included in each experiment.

Bioassay of AHL activity. The autoinducer of the *rhl* QS system, C₄-HSL, was measured using the *rhlA* promoter-based *P. aeruginosa* strain PDO100(pKD-*rhlA*) (41). PDO100 is an *rhlI* mutant that is deficient in signal molecule C₄-HSL production and, consequently, lacks *rhlA* expression unless exogenous C₄-HSL is present in the medium. The plasmid pKD-*rhlA* in PDO100 carries the C₄-HSL-responsive *rhlA* promoter fused upstream of promoterless *luxCDABE*. The light production of the reporter is proportional to the amount of C₄-HSL present in the medium. In the assay, 2 µl of a test bacterial culture (OD₆₀₀ = 1.0) of wild-type PAO1, PAO1 with DMSO, and the *rhlI* mutant (PDO100) or LB medium alone was inoculated onto the seeded bioassay plate. These plates were incubated at 37°C for 24 h. A bright halo zone around the bacterial colonies indicates AHL activity. The assays were also carried out in LB broth using the same reporter fusions. Briefly, overnight cultures of the reporter strains were diluted 1:300 in LB medium, and 90 µl of these solutions was added to the wells of a 96-well plate. Ten microliters of filter-sterilized culture supernatants containing either DMSO (2%, vol/vol) or LB medium (as a control) was added to the wells, the luminescence (measured in cps) and OD₆₀₀ values were measured every half hour for a total of 24 h using a Victor³ multilabel plate reader (Perkin-Elmer), and the relative levels of C₄-HSL were calculated from the maximal cps values.

Animal studies using a mouse model of burn wound infection. The experimental animals consisted of female C57BL/6J mice (age, 8 to 10 weeks), which were purchased from the Experimental Animal Center of the Fourth Military Medical University, Xi'an, Shaanxi, China. The animals were housed in ventilated cages in a pathogen-free facility operated with 12-h light and 12-h dark cycles at 23°C (standard deviation [SD], 2°C) and 30% to 60% humidity. All mice were allowed free access to a standard laboratory rodent chow and water. Individual animals were anesthetized intraperitoneally with 1% pentobarbital (50 mg/kg of body weight) and subjected to surgical procedures or sample collection. The experimental protocols were approved by the Experimental Animal Committee of the Fourth Military Medical University.

The effect of DMSO on *P. aeruginosa* PAO1 pathogenicity was examined by using the mouse of model burn wound infection as described previously with minor modifications (42, 43). The mice were anesthetized and shaved on the back. Thermal injury was induced by scalding about 10% of the body surface, and the mice were burned throughout the full thickness of skin (tertiary burn) using 95°C to 98°C water steam for 5 s. Immediately after they were burned, the mice were administered 0.3 ml of sterile saline intraperitoneally to prevent dehydration. PAO1 was subcultured and grown at 37°C to an optical density at 600 nm of approximately 0.9. Cells were then pelleted and serially diluted in saline. Sterilized filter paper was placed on the burn eschar, and 50 µl of a bacterial suspension was allowed to permeate the filter paper.

For dissemination tests, 3 groups of mice with 9 mice per group were used. The *P. aeruginosa* concentration in the inoculant suspension used on the burn eschar was 1 × 10⁵ CFU/ml. The mice were randomly divided into a DMSO treatment group, an antibiotic treatment group (2% silver sulfadiazine cream), and a negative-control group (saline). Administration of drugs or saline started at 24 h postinfection. Two hundred microliters of a 10% DMSO solution in 0.9% saline was applied to the burned skin of animals in the DMSO treatment group. Animals in the antibiotic group were given 200 µl of silver sulfadiazine at 2% (wt/vol), and animals in the negative-control group were given 200 µl of saline. The administration of drugs and saline was carried out twice a day for 3 days.

At 3 days after the burn and infection, the mice were euthanized by intracardial injection of 0.2 ml of pentobarbital. The livers of the animals in the negative-control, antibiotic-treated, and DMSO-treated groups were removed, weighed, and homogenized in phosphate-buffered saline (44). A 100-µl aliquot of each homogenate was plated on LB agar plates to determine the bacterial load after the burn and infection. The number of

FIG 1 Inhibition of pyocyanin production by DMSO in *P. aeruginosa*. (a) Color changes of *P. aeruginosa* cultures in the presence of DMSO, a reflection of the effect of DMSO on phenazine production. (b) Changed expression of *phzA1* and *phzA2* on solid medium. Reporter strains were plated on soft-top LB agar (0.7%, wt/vol) supplemented or not supplemented with DMSO, and imaging was done using an LAS3000 imaging system. (c, d) Expression of *phzA1* (c) and *phzA2* (d) in LB broth supplemented or not supplemented with 2% (vol/vol) DMSO. These experiments were repeated three times, and the data shown are representative of those from similar experiments. Relative luminescence unit (RLU), cps/OD₆₀₀.

bacteria from each liver was calculated as the number of CFU per gram of tissue.

For lethality tests, the procedures used to burn and infect the mice were the same as those stated above, except that a different inoculation dosage of *P. aeruginosa* was used. Sterilized filter paper was placed on the burn eschar, and 50 μ l of a *P. aeruginosa* suspension at 1×10^8 CFU/ml was allowed to permeate the filter paper.

The experiment was performed on four groups of mice with 11 mice per group. The mice were randomly divided into a group treated with a low dose of DMSO, a group treated with a high dose of DMSO, an antibiotic control group (imipenem), and a negative-control group (saline). In accordance with a previous study (45), the DMSO concentration used for the low-dose group was limited to 1.5 ml/kg of body weight, and the concentration used for the high-dose group was two times that used for the low-dose group. Accordingly, animals in the low-dose DMSO treatment group and the high-dose DMSO treatment group received a 10% DMSO solution in saline intraperitoneally at 15 μ l/g and 30 μ l/g DMSO per day, respectively. Animals in the antibiotic control group were injected with imipenem at 200 mg/kg daily. The final volumes of the injections were adjusted to 0.4 ml, and animals in the negative-control group were given 0.4 ml of saline. The administration of drugs and saline started at 2 h postinfection and was carried out twice a day for 5 days, and mortality was recorded during this period of time.

Statistical analysis. Student's *t* test was used to analyze the data from all the experiments except those from the animal studies. The Mann-Whitney *U* test was used for analysis of nonparametric data to determine the significance of the differences in the numbers of CFU between two groups of mice. The Kaplan-Meier test was used to determine the significance of the differences in mortality between the different mouse groups. All statistical analyses were performed by SPSS software (version 16.0). A *P* value of <0.05 was considered statistically significant.

RESULTS

Effect of DMSO on expression of QS-related genes and global regulators. It was observed during a QSI screening that the production of pigmented phenazine by *P. aeruginosa* PAO1 was greatly reduced in the presence of 2% (vol/vol) DMSO alone, resulting in a clear difference in the color of the cultures (Fig. 1a). To examine whether such an effect was related to bacterial growth, the impact of DMSO on the growth of *P. aeruginosa* PAO1 was determined with increasing concentrations of DMSO. The result indicated that there was no difference in PAO1 growth in the presence of DMSO at concentrations below 2.5% (vol/vol) (see Fig. S1 in the supplemental material).

The impact of 2% (vol/vol) DMSO on the expression of phen-

TABLE 2 Effect of DMSO on expression of virulence-related genes in *P. aeruginosa*

Virulence factor	Function	Fold change of gene expression level ^a
<i>lasI</i>	Autoinducer synthesis protein LasI	−1.5*
<i>lasB</i>	Elastase LasB synthesis	−5.3**
<i>lasA</i>	LasA protease synthesis	−2.9*
<i>rhlA</i>	Rhamnolipid synthesis	−20.1**
<i>rhlI</i>	Autoinducer synthesis protein RhlI	−1.4*
<i>phzA1</i>	Phenazine biosynthesis protein	−2.6*
<i>phzA2</i>	Phenazine biosynthesis protein	−4.7**
<i>pqsA</i>	Anthranilate coenzyme A ligase	+2.1*
<i>pqsR</i>	Transcriptional regulator PqsR	−1.8*
<i>pilG</i>	Twitching motility protein PilG	−1.5*
<i>flhC</i>	Flagellin type B	−1.8*
<i>flhA</i>	Flagellar biosynthesis protein FlhA	−3.1*
<i>exoS</i>	Exoenzyme S (ADP-ribosyltransferase)	+1.7, ^b +7.5**
<i>exoY</i>	Adenylate cyclase ExoY	+1.8, ^b +3.2*
<i>exoT</i>	Exoenzyme T	+1.5, ^b +8.1**
<i>gacA</i>	Response regulator GacA	−4.4**
<i>rpoS</i>	Sigma factor RpoS	−3.0*

^a The gene expression data were normalized by bacterial growth (OD₆₀₀). Student's *t* test was used to analyze the data. −, repression of gene expression by DMSO; +, activation of gene expression by DMSO. * and **, statistically very significant difference ($P < 0.01$) and statistically highly significant difference ($P < 0.001$), respectively.

^b Changes under calcium-depleted, T3SS-inducing conditions.

azine biosynthesis gene clusters, *phzA1* and *phzA2*, was then determined. As shown in Fig. 1b to d, DMSO significantly repressed the expression of *phzA1* and *phzA2*, which probably explains the reduced level of phenazine production with the presence of DMSO in the medium.

Since phenazine compounds, including pyocyanin, are products controlled by the QS system in *P. aeruginosa* (46, 47), we tested whether the expression of genes associated with the QS system were influenced by DMSO. The results (Table 2) indicate that the expression of QS-related genes, including *lasI*, *lasR*, *rhlI*, *rhlR*, *lasB*, and *rhlA*, was inhibited by DMSO. Examination of the effect of DMSO on other regulatory systems, such as the GacA-GacS two-component system and the *Pseudomonas* quinolone signal (PQS) system, which also play important roles in regulating phenazine production (48, 49), was also carried out. In addition to the AHL-mediated QS system, DMSO inhibited the expression of *gacA*, *rpoS*, *pqsR*, and *vfr* to different extents, while it activated the expression of *pqsA*, which is involved in the synthesis of the PQS, and *exoS*, *exoT*, and *exoY*, which are the effector genes of the type III secretion system (T3SS) (Table 2). The T3SS injects the effectors into host cells, where the effectors exert different effects that promote infection and suppress the host immune response. The activation of *exoS*, *exoT*, and *exoY* was not significant under T3SS-inducing conditions but was more pronounced under noninducing condition. These results demonstrate that DMSO could influence the expression of some of the important global regulators and the effector genes of the T3SS in *P. aeruginosa*, together with the QS system.

Decreased production of QS-regulated extracellular virulence factors and biofilm formation by DMSO. The two zinc metalloproteases LasB and LasA are believed to play important roles in *P. aeruginosa* pathogenesis (50). Moreover, the involvement of rhamnolipids, synthesized by enzymes encoded by *rhlA*, in biofilm formation is well documented. They are involved in the

maintenance of open channels by affecting cell-cell interactions and in the attachment of bacterial cells to surfaces (51). As shown in Fig. 2 and Table 2, DMSO dramatically reduced the expression of *rhlA*, *lasB*, and *lasA*. To confirm such effects, the levels of production of elastase, LasA protease, and rhamnolipids in LB broth supplemented or not supplemented with DMSO were compared.

In agreement with the gene expression data, there were significant decreases in the levels of production of rhamnolipids ($P = 0.0037$), elastase ($P = 0.0088$), and LasA protease ($P = 0.00056$) in the presence of 2% (vol/vol) DMSO (Fig. 3a to c).

Biofilm formation, a QS-controlled phenomenon in *P. aeruginosa*, prompts increased antibiotic resistance and contributes to the severity of the infection in the lungs of patients with cystic fibrosis (52). Since DMSO repressed a range of QS-controlled

FIG 2 Repression of *rhlA* (a), *lasB* (b), and *lasA* (c) expression by DMSO. The experiments were repeated at least three times. The results represent the means from triplicate experiments, and the error bars indicate standard deviations. Relative luminescence unit (RLU), cps/OD₆₀₀.

FIG 3 Decreased production of virulence factors in the presence of DMSO. The production of rhamnolipids (a), LasB protease (b), and LasA protease activity (c) and biofilm formation (d) are shown. LasA protease activity was determined by measuring the ability of culture supernatants to lyse boiled *S. aureus* cells. LB, LB + DMSO, PAO1, and PAO1 + DMSO, LasA protease activity in LB medium (negative control 1), LB medium with DMSO (negative control 2), PAO1 culture LB medium supernatant, and PAO1 culture LB medium with DMSO supernatant, respectively. Results represent the means $\pm$ SD values from three independent experiments performed in triplicate. Student's *t* test was used to analyze the data. **, statistically very significant differences ($P < 0.01$).

genes which are involved in biofilm formation, we tested whether DMSO inhibited biofilm formation. As shown in Fig. 3d, the presence of DMSO greatly repressed the biofilm formation of *P. aeruginosa* ($P = 0.00027$).

The impact of DMSO on virulence was through the *rhl* system. It is known that many of the virulence factors affected by DMSO, including phenazine production and biofilm formation, are positively controlled by the C_4 -HSL-mediated *rhl* QS system. It is possible that the altered expression of the virulence factors caused by DMSO could have resulted from DMSO's inhibition of the *rhl* QS system. To test this possibility, we determined the impact of DMSO on the production of the homoserine lactone signal C_4 -HSL using an *rhlA* promoter-based *P. aeruginosa* reporter strain, PDO100(pKD-*rhlA*). The amount of C_4 -HSL in the culture supernatant in the presence and absence of DMSO was measured as the level of light production using this reporter system. As shown in Fig. 4, the level of C_4 -HSL in PAO1 was significantly reduced in the presence of DMSO ($P = 1.75 \times 10^{-8}$), which is consistent with the decreased gene expression at the transcriptional level. The results indicate that DMSO influences virulence factors probably by inhibiting the production of C_4 -HSL.

To further prove the connection between the *rhl* system and the effect of DMSO, we examined the impact of DMSO on the production of pyocyanin by addition of exogenous C_4 -HSL. As shown in the Fig. 5a, the addition of C_4 -HSL could negate DMSO's repression of pyocyanin production in comparison with the level of production for the wild type ($P = 0.00013$). We further

assessed biofilm formation in *rhlI* mutant strain PDO100 (deficient in C_4 -HSL production). As presented in Fig. 5b, in contrast to the findings for wild-type strain PAO1, no repression of biofilm formation was observed for PDO100 ($P = 0.2638$) in the presence of DMSO. The addition of extraneous C_4 -HSL elevated the level of biofilm formation of PDO100 to the wild-type level, and extraneous C_4 -HSL was able to restore the inhibitory effect of DMSO on PDO100 biofilm formation ($P = 0.00021$). Taken together, these results indicate that DMSO inhibited the production of virulence factors and biofilm formation, probably via the *rhl* system.

Activation of the effector genes of the type III secretion system by DMSO. It has been reported that the T3SS is negatively regulated by the *rhl* system (53). We determined whether DMSO could affect the expression of T3SS in *P. aeruginosa*. In contrast to the above-examined virulence factors, the presence of DMSO at 2% (vol/vol) activated the expression of *exoS*, *exoT*, and *exoY*, the effector genes of the T3SS (Table 2). Interestingly, DMSO treatment resulted in lower levels of activation of *exoS*, *exoT*, and *exoY* expression in the *rhlI* mutant background (PDO100) than in the wild-type strain PAO1 (data not shown), indicating that the activation of the T3SS by DMSO is partially dependent on the *rhl* QS system. Consistent with the activation of T3SS effectors, response regulator gene *gacA* of the GacA/S-RsmA regulatory pathway was repressed by the presence of DMSO (Table 2). It is known that the GacA/S two-component system negatively regulates RsmA synthesis, which is required for T3SS. Clearly, more than one pathway is involved in the effect of DMSO on T3SS.

FIG 4 Inhibition of C₄-HSL production by DMSO in PAO1. (a) The relative amount of C₄-HSL production by PAO1 in LB broth with and without the presence of DMSO. The amount of C₄-HSL in the culture supernatants was measured by determination of *rhlA* expression levels using the reporter strain PDO100 *rhlA* (39). LB medium and the culture supernatant of the C₄-HSL-nonproducing strain (*rhlI* deletion strain PDO100) were used as negative controls. Student's *t* test was used to analyze the data, and the means and standard deviations are shown. **, very significant difference ($P < 0.01$); ***, highly significant difference ($P < 0.001$). The experiments were repeated three times. (b) The AHL plate bioassay was performed using the reporter *P. aeruginosa* PDO100 pKD-*rhlA*. Two microliters of filter-sterilized culture supernatant was spotted on the plate seeded with the reporter. Fresh LB medium and the culture supernatant of C₄-HSL-nonproducing strain PDO100 were included as controls. A halo zone around the sample spot indicates the presence of C₄-HSL in the sample, and the size and intensity of the halo are proportional to the amount of C₄-HSL in the samples.

Treatment of *P. aeruginosa* infection by DMSO in a mouse model of burn wound infection. Based on the result that DMSO affected the production of a plethora of QS-related virulence factors at a noninhibitory concentration (2%, vol/vol), we employed a mouse model of burn wound infection to test DMSO's effect on *P. aeruginosa* pathogenicity *in vivo*. Two different animal studies were carried out, with one testing bacterial dissemination and the other testing lethality.

For the dissemination tests, the mice were randomly divided into a DMSO treatment group, an antibiotic treatment group (silver sulfadiazine), and a negative-control group (saline). Two hundred microliters of 10% DMSO solution in 0.9% saline was applied to the burned skin of animals in the DMSO treatment group at 24 h postinfection. Animals in the antibiotic treatment group were given 2% silver sulfadiazine cream, and animals in the negative-control group were given 200 μ l of saline. At 3 days after the

burn and infection, the number of bacteria from each liver was determined and calculated as the number of CFU per gram of tissue. As shown in Fig. 6, the bacterial loads in the DMSO- and silver sulfadiazine-treated groups were significantly lower than those in the untreated group. No significant difference between the DMSO-treated group and the silver sulfadiazine-treated group was observed. It is to be noted that a high concentration of DMSO (e.g., 10%) could inhibit *P. aeruginosa* growth (see Fig. S2 in the supplemental material). The application of DMSO at the burn infection site might affect bacterial growth. However, since DMSO was administered at 24 h postinfection and a relatively large number of *P. aeruginosa* cells was inoculated, such an effect probably represents a minor contributing factor.

To test the effect of DMSO on mouse survival after burn infection, four groups of mice ($n = 11$ per group) were burned and inoculated with approximately 5×10^6 CFU (50 μ l of *P. aerugi-*

FIG 5 The *rhl* system and effect of DMSO on pyocyanin production and biofilm formation. (a) Effect of DMSO on pyocyanin production with and without C₄-HSL (C₄). (b) Biofilm formation of PAO1, PDO100, and PDO100 with C₄-HSL. Student's *t* test was used to analyze the data, and the means and standard deviations are shown. *, statistically significant differences ($P < 0.05$); **, very significant differences ($P < 0.01$).

FIG 6 Effect of DMSO on bacterial load in the mouse model of burn wound infection determined by the dissemination test. A log number of CFU of zero indicates that no CFU were detected. The Mann-Whitney U test was used for analysis of nonparametric data to determine the significance of the differences in the numbers of CFU between two groups. *, significant difference from the saline-treated group ($P < 0.05$).

nosa suspension at 1×10^8 CFU/ml) of *P. aeruginosa*. The treatments started at 2 h postinfection, and the survival of the mice was followed for a total of 5 days. As shown in Fig. 7, the survival rate of the mice treated with a low dose of DMSO was significantly higher than that of the mice in the saline-treated group ($P = 0.021$). Similarly, the mice in the antibiotic treatment group had a significantly higher survival rate than those in the saline-treated group ($P = 0.002$). Enhanced survival was observed for mice treated with a high dose of DMSO in comparison with that ob-

TABLE 3 Effect of DMSO on antibiotic MICs against *P. aeruginosa* PAO1

DMSO concn (%)	MIC ^a (μg/ml)					
	CIP	CEF	MEM	CAR	CHL	TET
0	0.4	1.5	4	150	15	10
1	0.2	3	4	150	30	10
2	0.2	3	4	150	30	10

^a CIP, ciprofloxacin; CEF, ceftazidime; MEM, meropenem; CAR, carbenicillin; CHL, chloramphenicol; TET, tetracycline.

served for the mice in the saline-treated group. However, there was no statistically significant difference between the two DMSO-treated groups ($P > 0.05$). Taken together, the results suggest that DMSO could attenuate *P. aeruginosa* pathogenicity in the mouse model of burn wound infection and, hence, could potentially be used for the treatment of infections caused by *P. aeruginosa*.

Interaction of DMSO with antibiotics. It has recently been reported that DMSO affects antibiotic susceptibilities in *Escherichia coli* (54). To test whether DMSO interferes with the effect of conventional antibiotics, we tested the MICs of six antibiotics from different classes for *P. aeruginosa* in the presence of different concentrations of DMSO. The results are shown in Table 3. DMSO at 1% or 2% made *P. aeruginosa* slightly more sensitive to killing by ciprofloxacin, whose MIC decreased from 0.4 μg/ml to 0.2 μg/ml. However, the MICs of both chloramphenicol and ceftazidime increased 2-fold in the presence of DMSO. No changes to the MICs of meropenem, carbenicillin, or tetracycline were observed. It seems that the interaction of DMSO with antibiotics was antibiotic specific.

DISCUSSION

The success of *P. aeruginosa* as an opportunistic pathogen depends largely on its battery of virulence arsenals. In this study, we have shown that DMSO, an important polar aprotic solvent, was capable of affecting the expression of a plethora of QS-related genes at

FIG 7 Attenuation of *P. aeruginosa* pathogenicity by DMSO. The survival rates of the burned and infected mice are plotted. Mice in the control group were infected but treated with saline. The Kaplan-Meier test was used to determine the differences in the survival rate between groups of mice.

a noninhibitory concentration (2%, vol/vol). DMSO could significantly attenuate QS-controlled virulence factor production in *P. aeruginosa*, including biofilm formation and the production of pyocyanin, rhamnolipids, LasA protease, and elastase, all of which are known to be positively controlled by the *rhl* QS system (55). DMSO inhibited the transcription of the *rhlI* gene and reduced the level of C₄-HSL, and the effect of DMSO on QS-controlled virulence factors was diminished in the *rhlI* mutant background (PDO100). The addition of C₄-HSL to the culture of PDO100 could reverse the impact of DMSO on the expression of these virulence factors. These results indicate that the inhibitory effect of DMSO on *P. aeruginosa* QS-controlled virulence factors was mediated through the *rhl* system and the reduction of C₄-HSL by DMSO was the main cause of its impact on these virulence factors.

DMSO treatment activated the expression of *pqsA*, involved in the synthesis of PQS. A previous study reported that the transcription of *pqsA* is repressed by the *rhl* QS system, and *pqsABCDE* transcription increases accordingly in *rhlI* mutant strain PDO100 (56). It is possible that the activation of *pqsA* expression by DMSO was due to the inhibition of C₄-HSL by DMSO. The activation of T3SS effectors by DMSO can also be partially explained by the connection between the *rhl* system and the T3SS. However, the activation of *gacA*, *rpoS*, and *pqsR* by DMSO also probably accounts for the effect on the T3SS, since the T3SS is negatively regulated by GacA and RpoS (57, 58). Further investigation is required to understand the complete regulatory mechanisms involved in *P. aeruginosa* in relation to the effect of DMSO.

The finding in this study that DMSO (2%, vol/vol) exhibited significant antagonistic activities on the QS system is somewhat unexpected, considering that DMSO is frequently used as a solvent in screening for QS inhibitors. The QS system is an intriguing target for antimicrobial therapy, since it plays an essential role in regulating the expression of genes involved in pathogenicity and the formation of biofilms. Disruption of the QS signaling pathway is one promising way for controlling infections; the production and secretion of important virulence factors in pathogens, including *P. aeruginosa*, would be prevented by blocking QS. One of the best-studied examples of QS inhibitors is halogenated furanone compounds from a marine alga, *Delisea pulchra*. Several other compounds, such as the chemically modified QS inhibitor (QSI) furanone C-30 and ajoene from garlic, have also been identified, and they can attenuate infection by *P. aeruginosa* *in vivo* (59). The macrolide antibiotic azithromycin has also been revealed to interfere with the *P. aeruginosa* QS system to inhibit virulence factor production and biofilm formation (60, 61), and this finding holds great promise for macrolide therapy for chronic pulmonary infection in cystic fibrosis patients. The repression of the *rhl* system and its related virulence factors makes DMSO an ideal QSI candidate for the treatment of infections caused by *P. aeruginosa*.

Accordingly, we used a mouse model of a burn wound infection with *P. aeruginosa* to test whether DMSO may also have practical use of significance against *P. aeruginosa* infections owing to its QS antagonistic activities. The fact that DMSO significantly reduced the bacterial load and enhanced the survival rate of the burned mice infected with *P. aeruginosa* is remarkable. DMSO has been used to treat many illnesses in humans, including dermatologic diseases (21), gastrointestinal disorders (24–27), wound healing (6), pulmonary conditions (28, 29), and interstitial cystitis (IC) (62). However, its usefulness has, in most cases, been controversial, and the only FDA-approved use is for the treatment of

interstitial cystitis. In addition, the pharmacological mechanisms of DMSO in almost all the cases are unknown. It is not known whether the usefulness, if any, of the DMSO treatment in the above-mentioned conditions is related to its effect on the bacteria potentially involved. Interestingly, it has recently been reported that DMSO is effective for preventing *Acanthamoeba* keratitis if it is included in contact lens disinfectants (63). It has also been shown that the reduced product of DMSO, dimethyl sulfide, is able to inhibit *Salmonella* host cell invasion and the expression of *hilA* and pathogenicity island-associated genes (64). While the increased survival rate of the animals may have been attributable to the decreased level of virulence factor production in the DMSO-treated animals, the reason for the decreased bacterial load in the liver of the DMSO-treated animals is not straightforward, considering that DMSO has no bactericidal or growth-inhibiting effect on *P. aeruginosa* at the concentrations used in this study. We speculate that the effect of DMSO as a QS inhibitor on *P. aeruginosa* virulence factors probably made the pathogen less pathogenic or even nonpathogenic and, hence, allowed the host immune system to rid itself of the pathogen or shifted the balance between the pathogen and the host defense mechanisms.

When the results obtained with DMSO treatment were compared with those obtained with imipenem treatment, the DMSO treatment was not as effective as the antibiotic treatment. Also, there was no significant increase in the survival rate for the higher-dose DMSO treatment group compared with that for the lower-dose DMSO treatment group. Considering the effect of DMSO on T3SS effectors, the maximal efficacy of DMSO may require an optimal concentration of DMSO to be administered. Besides the inhibitory effect on the virulence factors of *P. aeruginosa* *in vivo*, adverse effects of DMSO treatment on other virulence factors may be present. However, compared to the effect of DMSO on the T3SS under laboratory conditions, the effect of DMSO on the T3SS under T3SS-inducing conditions, which are more similar to the conditions found in the host, was minimal. The bigger effect on the T3SS under laboratory conditions might be due to other chemical properties of DMSO, such as its nucleophilic characteristic that alters the culture conditions.

It is conceivable that DMSO could best be used in combination with antibiotics. However, possible interactions between DMSO and antibiotics also need to be addressed. It has recently been reported that DMSO inhibits the action of some reactive oxygen species-dependent antibiotics against *Escherichia coli* (54). In light of this report, we have tested the effect of DMSO on the efficacy of six different antibiotics against *P. aeruginosa*. Different from what was reported for *E. coli*, DMSO at 1% or 2% made *P. aeruginosa* slightly more sensitive to killing by ciprofloxacin (the MIC decreased from 0.4 µg/ml to 0.2 µg/ml) but slightly less sensitive to killing by chloramphenicol and ceftazidime (2-fold increase). No changes in the MICs of meropenem, carbenicillin, or tetracycline were observed. It seems that the interaction of DMSO with antibiotics could be both antibiotic and bacterium species specific. Such an effect should be considered if both DMSO and antibiotics are used at the same time.

The mouse model of burn wound infection used in this study is, in fact, a sepsis model. With a high dose of *P. aeruginosa*, the burn and *P. aeruginosa* infection induced severe sepsis, which progressed rapidly into the systemic inflammatory response syndrome and multiple organ dysfunction syndrome (MODS) in mice (43). The ability of DMSO to lower bacterial loads when

applied topically and enhance the survival rate when injected intraperitoneally indicates that DMSO could potentially be used both as a systemic drug and as a topically administered agent for the treatment of *P. aeruginosa* skin infections after a burn.

DMSO is frequently used as a solvent in drug discovery research. DMSO's impact on the virulence factors of bacterial pathogens complicates its usage as a solvent in biological and medicinal studies. At the least, the use of caution over such an effect is warranted when DMSO is used as a vehicle in antibiotic research.

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REFERENCES

- Johansen HK, Kovesi TA, Koch C, Corey M, Høiby N, Levison H. 1998. *Pseudomonas aeruginosa* and *Burkholderia cepacia* infection in cystic fibrosis patients treated in Toronto and Copenhagen. *Pediatr Pulmonol* 26:89–96. [http://dx.doi.org/10.1002/\(SICI\)1099-0496\(199808\)26:2<89::AID-PPUL3>3.0.CO;2-C](http://dx.doi.org/10.1002/(SICI)1099-0496(199808)26:2<89::AID-PPUL3>3.0.CO;2-C).
- Lyczak JB, Cannon CL, Pier GB. 2002. Lung infections associated with cystic fibrosis. *Clin Microbiol Rev* 15:194–222. <http://dx.doi.org/10.1128/CMR.15.2.194-222.2002>.
- Fuqua C, Parsek MR, Greenberg EP. 2001. Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. *Annu Rev Genet* 35:439–468. <http://dx.doi.org/10.1146/annurev.genet.35.102401.090913>.
- Miller MB, Bassler BL. 2001. Quorum sensing in bacteria. *Annu Rev Microbiol* 55:165–199. <http://dx.doi.org/10.1146/annurev.micro.55.1.165>.
- Schuster M, Sexton DJ, Diggle SP, Greenberg EP. 2013. Acyl-homoserine lactone quorum sensing: from evolution to application. *Annu Rev Microbiol* 67:43–63. <http://dx.doi.org/10.1146/annurev-micro-092412-155635>.
- Duimel-Peeters IGP, Houwing RH, Teunissen CP, Berger MPF, Snoeckx LHEH, Halfens RJG. 2003. A systematic review of the efficacy of topical skin application of dimethyl sulfoxide on wound healing and as an anti-inflammatory drug. *Wounds* 15:361–370.
- Schuster M, Lostroh CP, Ogi T, Greenberg E. 2003. Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. *J Bacteriol* 185:2066–2079. <http://dx.doi.org/10.1128/JB.185.7.2066-2079.2003>.
- Wagner VE, Bushnell D, Passador L, Brooks AI, Iglewski BH. 2003. Microarray analysis of *Pseudomonas aeruginosa* quorum-sensing regulons: effects of growth phase and environment. *J Bacteriol* 185:2080–2095. <http://dx.doi.org/10.1128/JB.185.7.2080-2095.2003>.
- Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Kumar N, Schembri MA, Song Z, Kristoffersen P. 2003. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *EMBO J* 22:3803–3815. <http://dx.doi.org/10.1093/emboj/cdg366>.
- Schuster M, Greenberg EP. 2006. A network of networks: quorum-sensing gene regulation in *Pseudomonas aeruginosa*. *Int J Med Microbiol* 296:73–81. <http://dx.doi.org/10.1016/j.ijmm.2006.01.036>.
- Passador L, Cook JM, Gambello MJ, Rust L, Iglewski BH. 1993. Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. *Science* 260:1127–1130. <http://dx.doi.org/10.1126/science.8493556>.
- Winson MK, Camara M, Latifi A, Foglino M, Chhabra SR, Daykin M, Bally M, Chapon V, Salmond G, Bycroft BW. 1995. Multiple *N*-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 92:9427–9431. <http://dx.doi.org/10.1073/pnas.92.20.9427>.
- Costerton JW, Stewart PS, Greenberg EP. 1999. Bacterial biofilms: a common cause of persistent infections. *Science* 284:1318–1322. <http://dx.doi.org/10.1126/science.284.5418.1318>.
- Sakuragi Y, Kolter R. 2007. Quorum-sensing regulation of the biofilm matrix genes (*pel*) of *Pseudomonas aeruginosa*. *J Bacteriol* 189:5383–5386. <http://dx.doi.org/10.1128/JB.00137-07>.
- Favre-Bonte S, Kohler T, Van Delden C. 2003. Biofilm formation by *Pseudomonas aeruginosa*: role of the C₄-HSL cell-to-cell signal and inhibition by azithromycin. *J Antimicrob Chemother* 52:598–604. <http://dx.doi.org/10.1093/jac/dkg397>.
- Kohler T, Curty LK, Barja F, Van Delden C, Pechere JC. 2000. Swarming of *Pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. *J Bacteriol* 182:5990–5996. <http://dx.doi.org/10.1128/JB.182.21.5990-5996.2000>.
- Zhu H, He CC, Chu QH. 2011. Inhibition of quorum sensing in *Chromobacterium violaceum* by pigments extracted from *Auricularia auricular*. *Lett Appl Microbiol* 52:269–274. <http://dx.doi.org/10.1111/j.1472-765X.2010.02993.x>.
- Bodini S, Manfredini S, Epp M, Valentini S, Santori F. 2009. Quorum sensing inhibition activity of garlic extract and *p*-coumaric acid. *Lett Appl Microbiol* 49:551–555. <http://dx.doi.org/10.1111/j.1472-765X.2009.02704.x>.
- Chifiriu MC, DiŃu LM, Oprea E, LiŃescu S, Bucur M, MăruŃescu L, Enache G, Saviuc C, Burlibas A, M, Trăistaru T. 2009. In vitro study of the inhibitory activity of usnic acid on dental plaque biofilm. *Roum Arch Microbiol Immunol* 68:215–222.
- Müh U, Schuster M, Heim R, Singh A, Olson ER, Greenberg EP. 2006. Novel *Pseudomonas aeruginosa* quorum-sensing inhibitors identified in an ultra-high-throughput screen. *Antimicrob Agents Chemother* 50:3674–3679. <http://dx.doi.org/10.1128/AAC.00665-06>.
- Lishner M, Lang R, Kedar I, Ravid M. 1985. Treatment of diabetic perforating ulcers (mal perforant) with local dimethylsulfoxide. *J Am Geriatr Soc* 33:41–43. <http://dx.doi.org/10.1111/j.1532-5415.1985.tb02858.x>.
- Kingery WS. 1997. A critical review of controlled clinical trials for peripheral neuropathic pain and complex regional pain syndromes. *Pain* 73:123–139. [http://dx.doi.org/10.1016/S0304-3959\(97\)00049-3](http://dx.doi.org/10.1016/S0304-3959(97)00049-3).
- Shirley SW, Stewart BH, Mirelman S. 1978. Dimethyl sulfoxide in treatment of inflammatory genitourinary disorders. *Urology* 11:215–220. [http://dx.doi.org/10.1016/0090-4295\(78\)90118-8](http://dx.doi.org/10.1016/0090-4295(78)90118-8).
- Salim A. 1992. Role of oxygen-derived free radical scavengers in the management of recurrent attacks of ulcerative colitis: a new approach. *J Lab Clin Med* 119:710–717.
- Salim A. 1992. Allopurinol and dimethyl sulfoxide improve treatment outcomes in smokers with peptic ulcer disease. *J Lab Clin Med* 119:702–709.
- Salim A. 1992. Oxygen-derived free-radical scavengers prolong survival in colonic cancer. *Chemotherapy* 38:127–134. <http://dx.doi.org/10.1159/000238952>.
- Salim AS. 1991. Protection against stress-induced acute gastric mucosal injury by free radical scavengers. *Intensive Care Med* 17:455–460. <http://dx.doi.org/10.1007/BF01690766>.
- Pepin JM, Langner RO. 1985. Effects of dimethyl sulfoxide (DMSO) on bleomycin-induced pulmonary fibrosis. *Biochem Pharmacol* 34:2386–2389. [http://dx.doi.org/10.1016/0006-2952\(85\)90799-3](http://dx.doi.org/10.1016/0006-2952(85)90799-3).
- Iwasaki T, Hamano T, Aizawa K, Kobayashi K, Kakishita E. 1994. A case of pulmonary amyloidosis associated with multiple myeloma successfully treated with dimethyl sulfoxide. *Acta Haematol* 91:91–94.
- Lu C, Mattson MP. 2001. Dimethyl sulfoxide suppresses NMDA- and AMPA-induced ion currents and calcium influx and protects against excitotoxic death in hippocampal neurons. *Exp Neurol* 170:180–185. <http://dx.doi.org/10.1006/exnr.2001.7686>.
- Duan K, Dammel C, Stein J, Rabin H, Surette MG. 2003. Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. *Mol Microbiol* 50:1477–1491. <http://dx.doi.org/10.1046/j.1365-2958.2003.03803.x>.
- Liang H, Li L, Kong W, Shen L, Duan K. 2009. Identification of a novel

- regulator of the quorum-sensing systems in *Pseudomonas aeruginosa*. FEMS Microbiol Lett 293:196–204. <http://dx.doi.org/10.1111/j.1574-6968.2009.01544.x>.
33. Essar D, Eberly L, Hadero A, Crawford I. 1990. Identification and characterization of genes for a second anthranilate synthase in *Pseudomonas aeruginosa*: interchangeability of the two anthranilate synthases and evolutionary implications. J Bacteriol 172:884–900.
 34. Kurachi M. 1958. Studies on the biosynthesis of pyocyanin. Isolation and determination of pyocyanin. Bull Inst Chem Res Kyoto Univ 36:163–173.
 35. Ohman D, Cryz S, Iglewski B. 1980. Isolation and characterization of *Pseudomonas aeruginosa* PAO mutant that produces altered elastase. J Bacteriol 142:836–842.
 36. Kessler E, Safrin M, Olson JC, Ohman DE. 1993. Secreted LasA of *Pseudomonas aeruginosa* is a staphylolytic protease. J Biol Chem 268:7503–7508.
 37. Pearson JP, Pesci EC, Iglewski BH. 1997. Roles of *Pseudomonas aeruginosa* las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. J Bacteriol 179:5756–5767.
 38. Guerra-Santos LH, Kappeli O, Fiechter A. 1986. Dependence of *Pseudomonas aeruginosa* continuous culture biosurfactant production on nutritional and environmental factors. Appl Microbiol Biotechnol 24:443–448.
 39. Koch AK, Kappeli O, Fiechter A, Reiser J. 1991. Hydrocarbon assimilation and biosurfactant production in *Pseudomonas aeruginosa* mutants. J Bacteriol 173:4212–4219.
 40. O'Toole GA, Kolter R. 2002. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol Microbiol 30:295–304.
 41. Duan K, Surette MG. 2007. Environmental regulation of *Pseudomonas aeruginosa* PAO1 Las and Rhl quorum-sensing systems. J Bacteriol 189:4827–4836. <http://dx.doi.org/10.1128/JB.00043-07>.
 42. Faezi S, Sattari M, Mahdavi M, Roudkenar MH. 2011. Passive immunisation against *Pseudomonas aeruginosa* recombinant flagellin in an experimental model of burn wound sepsis. Burns 37:865–872. <http://dx.doi.org/10.1016/j.burns.2010.12.003>.
 43. Li N, Hu X, Liu Y, Wang Y, Wang Y, Liu J, Cai W, Bai X, Zhu X, Han J, Hu D. 2013. Systemic inflammatory responses and multiple organ dysfunction syndrome following skin burn wound and *Pseudomonas aeruginosa* infection in mice. Shock 40:152–159. <http://dx.doi.org/10.1097/SHK.0b013e31829aef41>.
 44. Filiatrault MJ, Tomblin G, Wagner VE, Van Alst N, Rumbaugh K, Sokol P, Schwingel J, Iglewski BH. 2013. *Pseudomonas aeruginosa* PA1006, which plays a role in molybdenum homeostasis, is required for nitrate utilization, biofilm formation, and virulence. PLoS One 8:e55594. <http://dx.doi.org/10.1371/journal.pone.0055594>.
 45. Carletti F, Ferraro G, Rizzo V, Cannizzaro C, Sardo P. 2013. Antiepileptic effect of dimethyl sulfoxide in a rat model of temporal lobe epilepsy. Neurosci Lett 546:31–35. <http://dx.doi.org/10.1016/j.neulet.2013.04.031>.
 46. Latifi A, Winslow MK, Foglino M, Bycroft BW, Stewart GS, Lazdunski A, Williams P. 1995. Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in *Pseudomonas aeruginosa* PAO1. Mol Microbiol 17:333–343. http://dx.doi.org/10.1111/j.1365-2958.1995.mmi_17020333.x.
 47. Bianchi SM, Prince LR, McPhillips K, Allen L, Marriotti HM, Taylor GW, Hellewell PG, Sabroe I, Dockrell DH, Henson PW. 2008. Impairment of apoptotic cell engulfment by pyocyanin, a toxic metabolite of *Pseudomonas aeruginosa*. Am J Respir Crit Care Med 177:35–43. <http://dx.doi.org/10.1164/rccm.200612-1804OC>.
 48. Gallagher LA, McKnight SL, Kuznetsova MS, Pesci EC, Manoil C. 2002. Functions required for extracellular quinolone signaling by *Pseudomonas aeruginosa*. J Bacteriol 184:6472–6480. <http://dx.doi.org/10.1128/JB.184.23.6472-6480.2002>.
 49. Reimann C, Beyeler M, Latifi A, Winteler H, Foglino M, Lazdunski A, Haas D. 1997. The global activator GacA of *Pseudomonas aeruginosa* PAO positively controls the production of the autoinducer *N*-butyryl-homoserine lactone and the formation of the virulence factors pyocyanin, cyanide, and lipase. Mol Microbiol 24:309–319. <http://dx.doi.org/10.1046/j.1365-2958.1997.3291701.x>.
 50. Kharazmi A. 1989. Interactions of *Pseudomonas aeruginosa* proteases with the cells of the immune system. Antibiot Chemother 42:42–49. <http://dx.doi.org/10.1159/000417602>.
 51. Davey ME, Caiazza NC, O'Toole GA. 2003. Rhamnolipid surfactant production affects biofilm architecture in *Pseudomonas aeruginosa* PAO1. J Bacteriol 185:1027–1036. <http://dx.doi.org/10.1128/JB.185.3.1027-1036.2003>.
 52. Verstraeten N, Braeken K, Debkumari B, Fauvart M, Franssaer J, Vermant J, Michiels J. 2008. Living on a surface: swarming and biofilm formation. Trends Microbiol 16:496–506. <http://dx.doi.org/10.1016/j.tim.2008.07.004>.
 53. Bleves S, Soscia C, Nogueira-Orlandi P, Lazdunski A, Filloux A. 2005. Quorum sensing negatively controls type III secretion regulon expression in *Pseudomonas aeruginosa* PAO1. J Bacteriol 187:3898–3902. <http://dx.doi.org/10.1128/JB.187.11.3898-3902.2005>.
 54. Mi H, Wang D, Xue Y, Zhang Z, Niu J, Hong Y, Drlica K, Zhao X. 2016. Dimethyl sulfoxide protects *Escherichia coli* from rapid antimicrobial-mediated killing. Antimicrob Agents Chemother 60:5054–5058. <http://dx.doi.org/10.1128/AAC.03003-15>.
 55. Brint JM, Ohman DE. 1995. Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. J Bacteriol 177:7155–7163.
 56. McGrath S, Wade DS, Pesci EC. 2004. Dueling quorum sensing systems in *Pseudomonas aeruginosa* control the production of the *Pseudomonas* quinolone signal (PQS). FEMS Microbiol Lett 230:27–34. [http://dx.doi.org/10.1016/S0378-1097\(03\)00849-8](http://dx.doi.org/10.1016/S0378-1097(03)00849-8).
 57. Brencic A, McFarland KA, McManus HR, Castang S, Mogno I, Dove SL, Lory S. 2009. The GacS/GacA signal transduction system of *Pseudomonas aeruginosa* acts exclusively through its control over the transcription of the RsmY and RsmZ regulatory small RNAs. Mol Microbiol 73:434–445. <http://dx.doi.org/10.1111/j.1365-2958.2009.06782.x>.
 58. Hogardt M, Roeder M, Schreff AM, Eberl L, Heesemann J. 2004. Expression of *Pseudomonas aeruginosa* *exoS* is controlled by quorum sensing and RpoS. Microbiol 150:843–851. <http://dx.doi.org/10.1099/mic.0.26703-0>.
 59. Bjarnsholt T, Jensen PO, Rasmussen TB, Christophersen L, Calum H, Hentzer M, Hougen HP, Rygaard J, Moser C, Eberl L. 2005. Garlic blocks quorum sensing and promotes rapid clearing of pulmonary *Pseudomonas aeruginosa* infections. Microbiology 151:3873–3880. <http://dx.doi.org/10.1099/mic.0.27955-0>.
 60. Nalca Y, Jansch L, Bredenbruch F, Geffers R, Buer J, Haussler S. 2006. Quorum-sensing antagonistic activities of azithromycin in *Pseudomonas aeruginosa* PAO1: a global approach. Antimicrob Agents Chemother 50:1680–1688. <http://dx.doi.org/10.1128/AAC.50.5.1680-1688.2006>.
 61. Hoffmann N, Lee B, Hentzer M, Rasmussen TB, Song Z, Johansen HK, Givskov M, Høiby N. 2007. Azithromycin blocks quorum sensing and alginate polymer formation and increases the sensitivity to serum and stationary-growth-phase killing of *Pseudomonas aeruginosa* and attenuates chronic *P. aeruginosa* lung infection. Antimicrob Agents Chemother 51:3677–3687. <http://dx.doi.org/10.1128/AAC.01011-06>.
 62. Sant G. 1987. Intravesical 50% dimethyl sulfoxide (Rimso-50) in treatment of interstitial cystitis. Urology 29:17.
 63. Siddiqui R, Aqeel Y, Khan NA. 2016. The use of dimethyl sulfoxide in contact lens disinfectants is a potential preventative strategy against contracting *Acanthamoeba* keratitis. Cont Lens Anterior Eye 39:389–393. <http://dx.doi.org/10.1016/j.clae.2016.04.004>.
 64. Antunes LC, Buckner MM, Auweter SD, Ferreira RB, Lolic P, Finlay BB. 2010. Inhibition of *Salmonella* host cell invasion by dimethyl sulfide. Appl Environ Microbiol 76:5300–5304. <http://dx.doi.org/10.1128/AEM.00851-10>.
 65. Liang H, Li L, Dong Z, Surette MG, Duan K. 2008. The YebC family protein PA0964 negatively regulates the *Pseudomonas aeruginosa* quinolone signal system and pyocyanin production. J Bacteriol 190:6217–6227. <http://dx.doi.org/10.1128/JB.00428-08>.