Phytopathology Research

RESEARCH Open Access

First method for dissolving zinc thiazole and re-evaluation of its antibacterial properties against rice bacterial blight disease

Xian Chen[1†], Lan Zhou[2†], Pedro Laborda[3], Yancun Zhao[1], Kaihuai Li[1] and Fengquan Liu[1*]

Abstract

Rice bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most destructive diseases in rice-growing regions worldwide. Zinc thiazole is a novel bactericide and has been applied for BB control for 10 years. However, zinc thiazole is highly insoluble in water and in most organic solvents. In this work, we found for the first time that zinc thiazole can be dissolved in dimethyl sulfoxide (DMSO), and the solubility of zinc thiazole in DMSO is more than 20 mg/mL. Dissolved zinc thiazole at 25 μg/mL significantly inhibited the growth of *Xoo* by 58.81%. Interestingly, zinc thiazole at 25 μg/mL enhanced the cell division and altered the cell wall integrity of *Xoo*. The application of dissolved zinc thiazole at 100 μg/mL reduced the incidence of rice bacterial blight (BB) by providing 64.71% control efficacy, while zinc thiazole as suspension concentrate (SC) at 100 μg/mL only provided 43.42% control efficacy. Taken together, this study provides for the first time a method for dissolving zinc thiazole, and may help to better understand the antibacterial mechanism of zinc thiazole.

Keywords: Zinc thiazole, Solubility, Antibacterial effect, Rice bacterial blight, *Xanthomonas oryzae* pv. *oryzae*

Background

It has been speculated that human world population will reach 9.7 billion by 2050 (Sessitsch et al. 2018), which indicates that global food production will have to be more than doubled at that time (Green et al. 2018). Therefore, it is extremely necessary to explore new methodologies, tools and processes to support and improve crop production (Shunmugam et al. 2018). Plant diseases, mainly caused by bacterial (Mansfield et al. 2012), viral (Scholthof et al. 2011) and fungal pathogens (Dean et al. 2012), and parasitic nematodes (Jones et al. 2013), are the main limiting factors of crop yields (Anglin et al. 2018). For example, rice bacterial blight (BB) leads to leaf blight during the growing season, hindering photosynthesis and diminishing the production and quality of rice (Kim and Reinke 2019). Breeding resistant varieties are a crucial strategy to control BB diseases (Kim 2018). Due to the emergence of new physiological races of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), many resistant varieties succumb to disease after a few years of cultivation. Thus, BB disease management still relies largely on chemical control (Kennelly et al. 2007). For this reason, it is urgent to develop environment-friendly control strategies with higher efficacy and lower toxicity.

Zinc thiazole (2-amino-5-mercapto-1,3,4-thiadiazole) is a broad spectrum bactericide for controlling crop diseases, including rice bacterial blight (BB) (Zhang et al. 2013; Zhu et al. 2014; Chen et al. 2015), rice bacterial leaf streak (BLS) (Wei et al. 2007), bacterial leaf spot of *Euphorbia pulcherrima* (Li et al. 2008) and cucumber downy mildew (Hu et al. 2012). This zinc complex is an insoluble molecule and is commercially available as zinc thiazole suspension concentrate (SC) since 2007. The thiazole ring has been reported to be soluble in organic

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solvents (Kashyap et al. 2012). Thus, the zinc atom may be the main reason for the insolubility of zinc thiazole. Zinc thiazole is found safe to non-target organisms and easy to be degraded in soil (Wei et al. 2007, 2008). However, the insoluble property of zinc thiazole greatly limits its activity.

In this study, dimethylsulfoxide (DMSO) was found to be a suitable solvent to dissolve zinc thiazole. The antibacterial mechanism of zinc thiazole (in DMSO solvent) against *Xoo* strain PXO99 was investigated, and the protective and curative activities of zinc thiazole against rice bacterial blight were determined. Our results may provide more accurate and meaningful data for reassessing the effect of zinc thiazole in controlling plant bacterial diseases.

Results

DMSO is a suitable solvent for zinc thiazole

In order to find a suitable solvent for zinc thiazole, ten solvents were tested, including MeOH, EtOH, isopropanol (IPA), acetone, acetonitrile (ACN), ethyl acetate (EA), dimethylformamide (DMF), Tween80, DMSO and sterilized ddH_2O. As shown in Fig. 1a, zinc thiazole was insoluble in MeOH, EtOH, IPA, acetone, ACN, EA, DMF and ddH_2O. Meanwhile, zinc thiazole was partially soluble in Tween80 since the mixture solution turned dark yellow with precipitation. Surprisingly, the white powder corresponding to zinc thiazole completely disappeared in DMSO solvent, providing a solution with blight yellow color, which indicated that zinc thiazole is soluble in DMSO (20 mg/mL). To identify whether the structure of zinc thiazole was damaged by DMSO solvent, zinc thiazole, dissolved in DMSO, was analyzed by mass spectrometry (Fig. 1b). In this sense, a main m/z peak could be observed at 328.8702 Da, which was consistent with the expected molecular weight of zinc thiazole, $[M + H]^+ = 328.8750$ Da. The obtained result confirmed that the compound is dissolved in DMSO without any obvious degradation. As shown in Fig. 1b, the analysis of the ionic fragments by MS/MS spectrometry, found at 227.8658, 195.8938 and 131.9506 Da, confirmed that the m/z peak at 328.8702 Da corresponds to zinc thiazole.

Antimicrobial activity of DMSO-solved zinc thiazole and zinc thiazol suspension concentrate against PXO99

Zinc thiazole SC is a commercial bactericide and has been used for over 10 years since it was produced (Wei

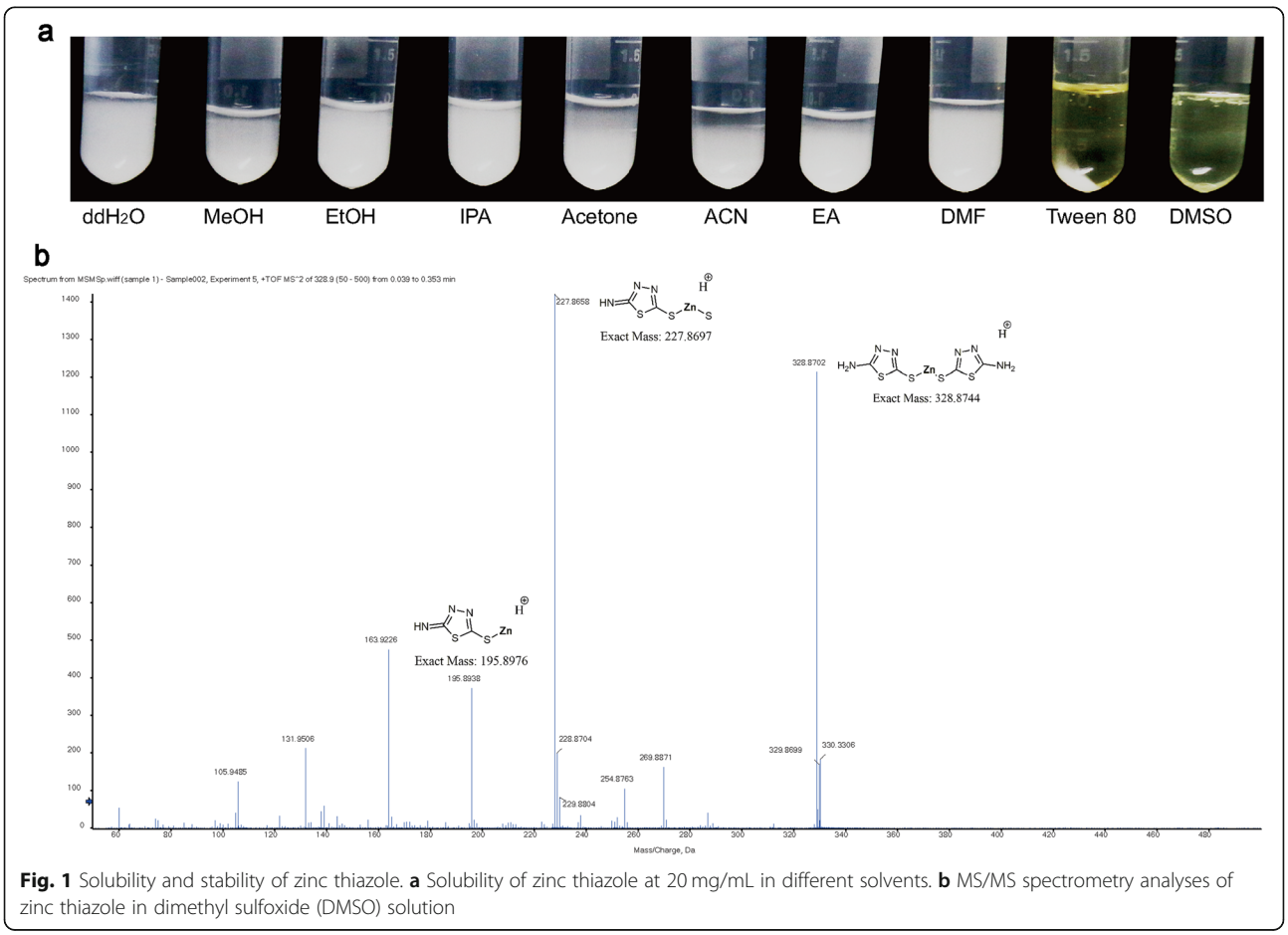

Fig. 1 Solubility and stability of zinc thiazole. **a** Solubility of zinc thiazole at 20 mg/mL in different solvents. **b** MS/MS spectrometry analyses of zinc thiazole in dimethyl sulfoxide (DMSO) solution

et al. 2007). To determine the different antibacterial activities of zinc thiazole in DMSO (20 μg/μL) and zinc thiazole SC (200 μg/μL), the diameter of the inhibition zone created by these two different dissolved forms of zinc thiazole was measured. As shown in Fig. 2a and b, both zinc thiazole SC and zinc thiazole in DMSO exhibited antibacterial activities against *Xoo*. The average diameter of the antibacterial inhibition zone produced by zinc thiazole in DMSO (20 μg/μL) was 1.6 ± 0.13 cm, while 1.4 ± 0.11 cm diameter was produced by zinc thiazole SC (200 μg/μL). DMSO showed no antibacterial activity to *Xoo*, indicating that zinc thiazol is the only agent responsible for the antibacterial activity. The results suggested that the antibacterial activity of soluble zinc thiazole at 20 μg/μL was similar to that of zinc thiazole SC at 200 μg/μL, which indicated that DMSO-solved zinc thiazol shows a higher antibacterial activity in comparison with zinc thiazol SC.

Zinc thiazole inhibits the proliferation of PXO99

Zinc thiazole showed relevant inhibitory activity against plant pathogenic bacterium PXO99. As shown in Table 1, the EC_{50} of zinc thiazole in the soluble state for *Xanthomonas campestris* pv. *campestris* (*Xcc*), *Erwinia pyrifoliae* (*Ep*), *Erwinia amylovora* (*Ea*), *Xanthomonas axonopodis* pv. *glycines* (*Xag*) and *Xoo* was 32.43, 7.83, 20.23, 31.77 and 10.98 μg/mL, respectively.

Proliferation of PXO99 at different zinc thiazole concentrations (0, 25 and 50 μg/mL) was assayed. DMSO was used as a negative control and ampicillin (50 μg/mL) was used as a positive control. It was observed that zinc thiazole at 25 μg/mL effectively inhibited PXO99 cell growth (Fig. 3a). The growth rate of PXO99 under zinc thiazole (25 μg/mL) treatment for 24 h was highly reduced in comparison to that of the control experiment (Fig. 3b). The inhibition effect of zinc thiazole on the growth of PXO99 cells was proportional to its concentration. In this sense, the complete growth inhibition of PXO99 was observed after incubation with zinc thiazole at 50 μg/mL, and similar results were observed using ampicillin. DMSO was found to produce no inhibitory effect on PXO99.

Zinc thiazole enhances cell division of PXO99

To investigate whether zinc thiazole inhibits bacterial proliferation by disrupting or inhibiting cell division, the mRNA expressions of nine cell-division-related genes in PXO99 challenged with zinc thiazole (25 μg/mL) were analysed by qRT-PCR. As shown in Fig. 4, the cell-division-related gene *Fst B* was 10-fold upregulated, whereas the cell-division-related genes *ZapA*, *FstZ*, *FstQ* and *FstL* were 2-fold upregulated when compared with the control experiment. The mRNA expressions of cell-division-related genes were only slightly changed in PXO99 treated with DMSO. Thus, our results indicated that the zinc thiazole treatment may lead to increased cell division in *Xoo*.

Zinc thiazole regulates cell morphology of PXO99

To further explore the antibacterial mechanism of zinc thiazole against *Xoo*, PXO99 was examined by transmission electron microscope (TEM) and scanning electron

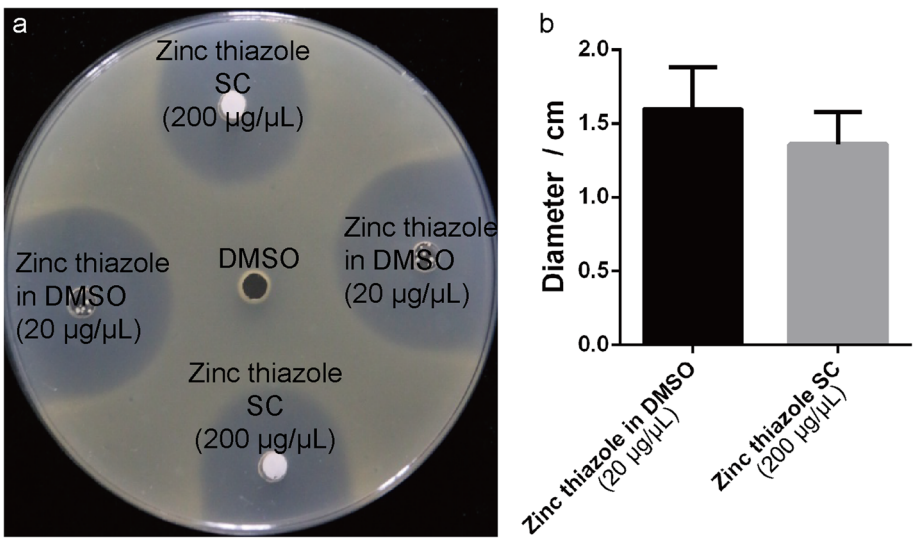

Fig. 2 Antibacterial activity of zinc thiazole against *Xoo*. **a** Antibacterial effect of zinc thiazole suspension concentrate (SC, 200 μg/μL) and DMSO-solved zinc thiazole (20 μg/μL) in Petri dishes containing *Xoo*. DMSO solvent (without dilution) added in the middle hole was used as a control. **b** Diameter of the inhibition zone produced by zinc thiazole SC and DMSO-solved zinc thiazole against *Xoo*. Error bars represent standard errors from three independent replicates

Table 1 Antibacterial toxicity of zinc thiazole against plant pathogens

Strain	Regression equation	EC_{50} (μg/mL)	R^2
Xcc	y = 5.2642x - 2.9542	32.43	0.94
Xag	y = 8.9474x - 8.4439	31.77	0.97
Ea	y = 4.9581x - 1.4752	20.23	0.95
Xoo	y = 3.1591x + 1.7121	10.98	0.91
Ep	y = 3.0745x + 2.2534	7.83	0.93

Xcc, Xanthomonas campestris pv. *campestris; Xag, Xanthomonas axonopodis* pv. *glycines; Ep, Erwinia pyrifoliae; Ea, Erwinia amylovora; Xoo, Xanthomonas oryzae* pv. *oryza*

microscope (SEM) to measure its ultrastructure under zinc thiazole treatment (25 μg/mL). Bacterial size and shape were easily distinguished by TEM and SEM (Fig. 5). It was observed that the cell wall of PXO99 in the absence of zinc thiazole showed complete cell shape (Fig. 5a, d), and similar results were detected when applying DMSO (Fig. 5c, f). When treated with zinc thiazole (25 μg/mL), the cell wall became uneven and some holes were found on the cell surface (red arrows) (Fig. 5b, e). Our results indicated that the antibacterial effects of zinc thiazole may be directly related to the observed incomplete cell wall in *Xoo*.

Protective and curative activities of zinc thiazole against BB

To evaluate the protective and curative activities of zinc thiazole against rice bacterial blight, rice leaves were sprayed with zinc thiazole before (protective) and after (curative) the inoculation of PXO99. As a positive control, the rice leaves were inoculated with the pathogen in the absence of zinc thiazole treatment. Then, the lesion length was measured at 7 days post inoculation (dpi). In the curative test, the average lesion lengths in the presence of 0, 12.5, 25, 50, 100 and 200 μg/mL of zinc thiazole were 7.84, 6.75, 5.04, 4.18, 2.3 and 0.4 cm, respectively (Fig. 6a, c). In the protective test, the average lesion lengths in the presence of 0, 12.5, 25, 50, 100 and 200 μg/mL of zinc thiazole were 5.58, 4.72, 3.42, 2.07, 1.74 and 0.69 cm, respectively (Fig. 6b, d). In the curative test, the control efficacy of zinc thiazole at 200 μg/mL was 94.89%, while the protective efficacy of zinc thiazole at 200 μg/mL was 87.65%. Zinc thiazole (containing 1% v/v DMSO) had no phytotoxic effect on the rice plants. These results indicated that zinc thiazole can supply both protective and curative activity against

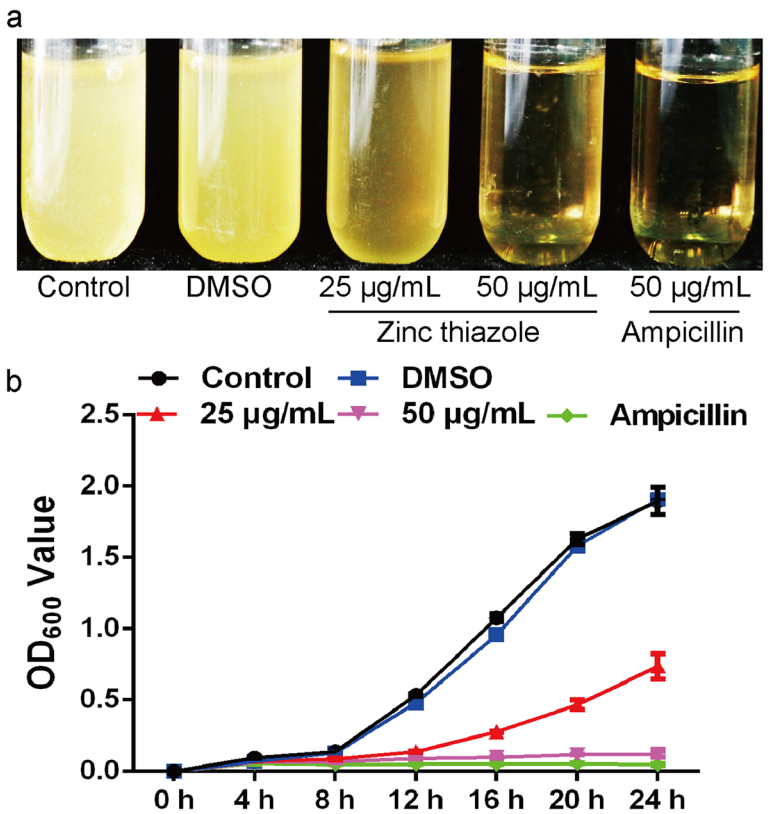

Fig. 3 Zinc thiazole inhibits the growth of *Xoo*. **a** Growth of PXO99 under zinc thiazole treatment. **b** Statistical analysis of growth curves of PXO99 treated with different concentrations of zinc thiazole and cultured for 24 h. Control indicates cell suspension of PXO99 (0 μg/mL zinc thiazole without DMSO). DMSO (0.125%) was used as a negative control, and ampicillin (50 μg/mL) was used as a positive control. Error bars represent standard errors from three independent replicates

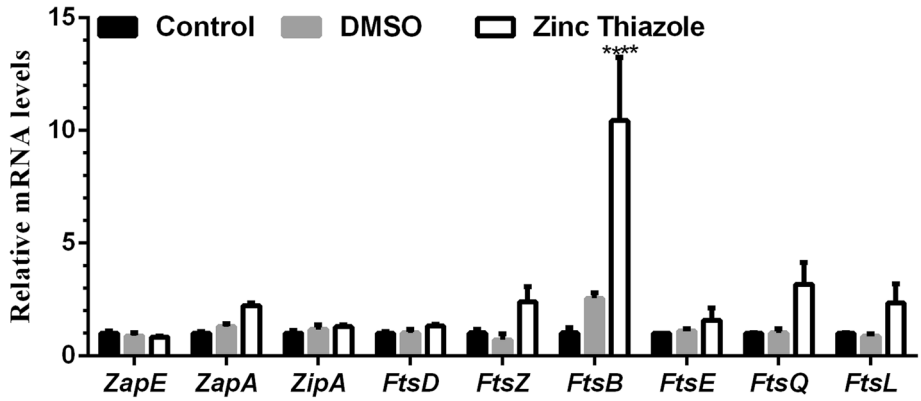

Fig. 4 qRT-PCR analysis of the mRNA expressions of cell-division-related genes in zinc thiazole-treated *Xoo*. PXO99 cultures were treated with zinc thiazole (20 μg/mL) in DMSO (0.125%) and DMSO (0.125%), respectively. Control indicates cell suspension of PXO99 (0 μg/mL zinc thiazole without DMSO). All groups were harvested at OD_{600} = 1.0. The total RNA of *Xoo* was extracted, and the relative gene expressions were quantified by qRT-PCR. The relative transcription levels were normalized to that of *RecA*. Error bars represent standard errors from three independent replicates. ****: $P < 0.0001$

BB disease, and the curative efficacy is higher than the protective efficacy.

To evaluate the different curative activities of dissolved zinc thiazole and zinc thiazole SC, rice leaves at 12 h post inoculation (hpi) with PXO99 were sprayed with dissolved zinc thiazole and zinc thiazole SC at 100 μg/mL. It was observed that the average lesion length in the control plants (treated with 0.1% DMSO) was 9.9 cm, whereas the lesion lengths after treatment with dissolved zinc thiazole and zinc thiazole SC were 3.5 and 5.6 cm, respectively (Fig. 7). The curative efficacies of dissolved zinc thiazole and zinc thiazole SC were 64.71% and 43.42%, respectively. Thus, our results indicated that dissolved zinc thiazole can provide higher curative activity than zinc thiazole SC.

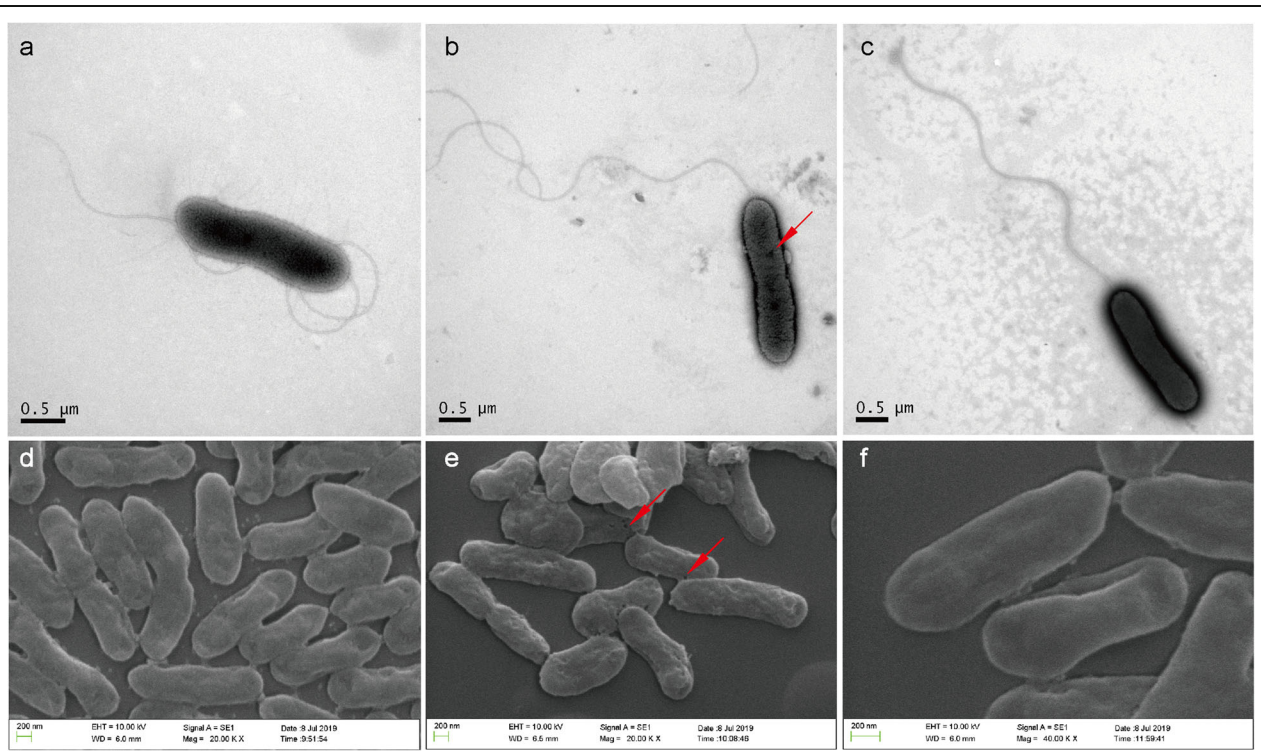

Fig. 5 Cell morphology of *Xoo* strain PXO99 observed with TEM (**a**, **b** and **c**) and SEM (**d**, **e** and **f**). **a**, **d** Non-treated PXO99 cells (0 μg/mL zinc thiazole). **b**, e PXO99 cells treated with zinc thiazole (25 μg/mL) in 0.125% DMSO. **c, f** PXO99 cells treated with 0.125% DMSO. Arrows indicate the incomplete cells

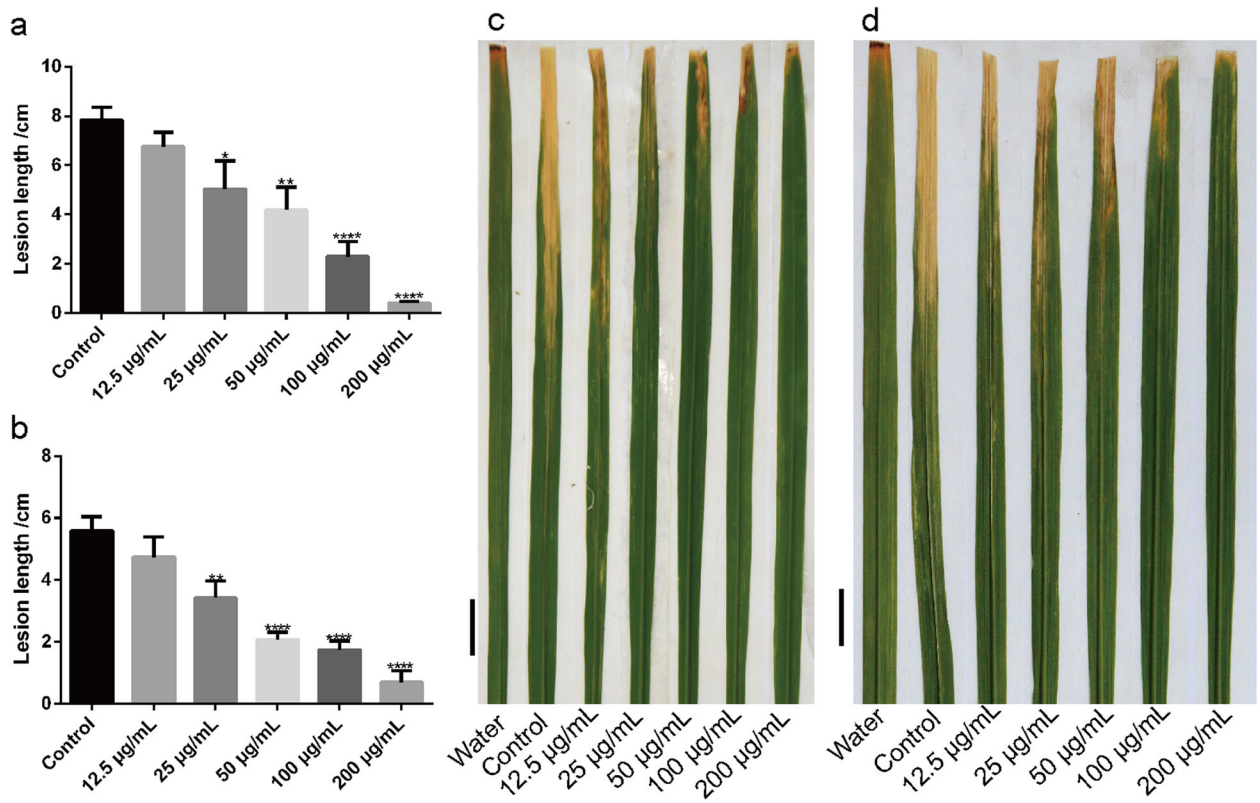

Fig. 6 Protective and curative activities of zinc thiazole against rice bacterial blight. **a** Lesion length (cm) of PXO99 in rice leaves treated with zinc thiazole: curative test at 7 dpi. Rice leaves were inoculated with *Xoo* and were sprayed with different concentrations of zinc thiazole (0, 12.5, 25, 50, 100 and 200 μg/mL) at 12 hpi. **b** Lesion length produced by PXO99 in rice leaves treated with zinc thiazole: protective test at 7 dpi. Rice leaves were sprayed with different concentrations of zinc thiazole (0, 12.5, 25, 50, 100 and 200 μg/mL) and were inoculated with *Xoo* after 12 h. **c** Symptom development of *Xoo*-inoculated leaves in the zinc thiazole curative test at 7 dpi. **d** Symptom development of *Xoo*-inoculated leaves in the zinc thiazole protective test at 7 dpi. Bar = 1 cm. *Xoo*-inoculated leaves were used as a positive control, and leaves treated with water (containing 0.1% DMSO) were used as a negative control. Error bars represent standard errors from three independent replicates. *: $P < 0.05$; **: $P < 0.01$; ****: $P < 0.0001$

Discussion

Chemical control and resistance varieties are the major ways to ensure crop production (Bartlett et al. 2002; Hirooka and Ishii 2013; Chukwu et al. 2019). Zinc thiazole is a novel bactericide and has been widely used for the control of plant bacterial diseases over 10 years (Wei et al. 2007). Zinc thiazole belongs to the family of thiazole ring derivatives (Mishra et al. 2017). However, zinc thiazole was reported to be an insoluble compound and the zinc thiazole suspension concentrate (SC) is the only commercial form that has been found on the market since 2007. Our research group succeeded for the first time in dissolving zinc thiazole in a solvent, DMSO, without affecting its structure.

It was reported that the EC_{50} of zinc thiazole SC to *Xoo* was 94.83 μg/mL (Zhu et al. 2014). In our study, the EC_{50} of zinc thiazole in the soluble state to *Xoo* was 10.98 μg/mL, indicating that the inhibitory activity of zinc thiazole in the soluble state was about 9 times higher than that in SC form. It was found that the production of extracellular polysaccharide (EPS) in *Xoo* was reduced more than 10% when challenged with zinc thiazole in SC form at 90 μg/mL (Liang et al. 2015), which may be due to the reduced growth of the bacterium. Previous studies indicated that the antibacterial effects of thiazole derivatives could be a result of anchoring to membrane-targeting cationic ligand toward bacteria (Dai et al. 2017), inhibiting the activities of enzyme and protein (Nakada et al. 2010) and triggering the aggregation of bacteria quickly and then killing them (Mohammad et al. 2015). Here, it was detected that zinc thiazole is also able to alter the cell wall of *Xoo*. Although several advances were reported during last years, the detailed antibacterial mechanism of zinc thiazole needs to be further explored.

Cell division plays many crucial roles in bacterial proliferation, and was shown to be regulated by Z-ring related genes *ZapE* and *FtsZ* by anchoring the ring at the

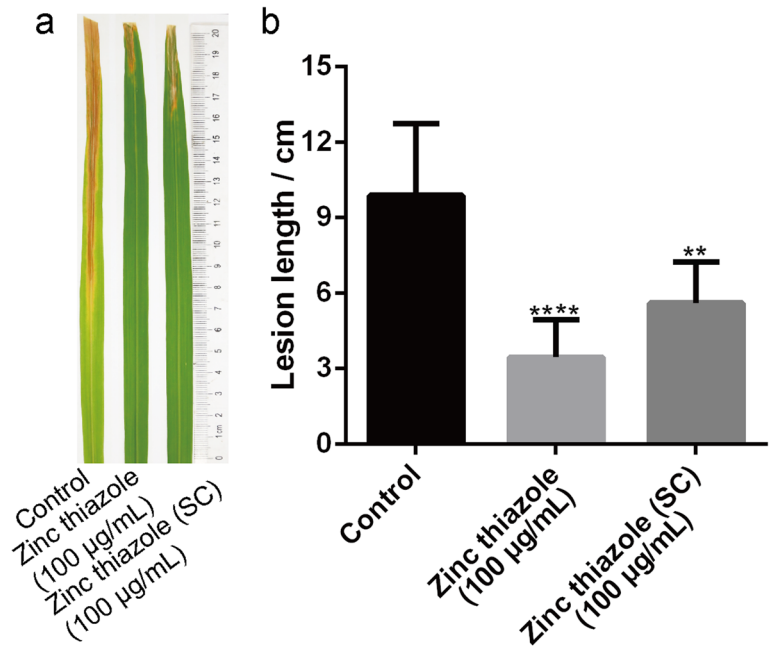

Fig. 7 Curative activities of dissolved zinc thiazole and zinc thiazole SC against rice bacterial blight. **a** Symptom development of *Xoo*-inoculated leaves after treatment with dissolved zinc thiazole and zinc thiazole SC: curative test at 7 dpi. Rice leaves were inoculated with *Xoo* and were sprayed with dissolved zinc thiazole (100 μg/mL) and zinc thiazole SC (100 μg/mL) at 12 hpi. Leaves were sprayed with 0.1% DMSO as a control experiment. **b** Lesion length (cm) of PXO99 in rice leaves treated with 0.1% DMSO, dissolved zinc thiazole and zinc thiazole SC. Error bars represent standard errors from three independent replicates. **: $P < 0.01$; ****: $P < 0.0001$

cytoplasmic membrane (Adams and Errington 2009). Then, a macromolecular complex is formed in the middle of the cell where *FtsB*, *FtsQ* and *FtsL* play essential roles (Villanelo et al. 2011). In our work, *ZapA*, *FtsZ*, *FtsB*, *FtsQ* and *FtsL* were upregulated in the presence of zinc thiazol, suggesting that zinc thiazole enhances the cell division.

Chemical bactericides would lose the ability to protect plants when mutations in bacteria lead to their resistance to these drugs (Elad et al. 1992). For example, bismerthiazol has been widely used in the control of rice bacterial diseases in China since the 70s (Dai et al. 2015). However, bismerthiazol-resistant *Xoo* strains have been detected in many rice-growing regions in China (Zhu et al. 2013). Both zinc thiazole and bismerthiazol are thiazole bactericides. It was reported a cross-resistance between zinc thiazole and bismerthiazol in *Xoo* (Zhu et al. 2014). In fields, zinc thiazole in SC form was shown to provide higher control efficacy against BB than bismerthiazol at the same concentration (Chen et al. 2015). Zinc thiazole in SC form, applied at 300 μg/mL, provided 64.8% control efficacy against BB (Chen et al. 2015), while zinc thiazole in the soluble state at 200 μg/mL provided 94.89% control efficacy. This result indicated that zinc thiazole in the soluble state achieved a better efficacy in comparison to that detected for the SC form. Therefore, zinc thiazole in the soluble state may be an interesting alternative for the management of BB and other plant bacterial diseases.

Conclusions

In summary, we developed an efficient strategy to dissolve zinc thiazole in DMSO without affecting its structure. The results of our study suggested that DMSO-solved zinc thiazole exhibited a higher antibacterial effect on the proliferation of *Xoo* and provided a better efficacy for controlling BB. Moreover, it was found that zinc thiazole is able to alter the cell wall morphology of *Xoo*. Thus, this study provides accurate and meaningful data for the reassessment of the control effect of zinc thiazole on plant bacterial diseases.

Methods

Bacterial strains

X. campestris pv. *campestris* (*Xcc*), *X. axonopodis* pv. *glycines* (*Xag*), *E. pyrifoliae* (*Ep*), *E. amylovora* (*Ea*) and *Xoo* strain PXO99 were grown at 28 °C in nutrient broth (NB) medium (3 g/L beef extract, 1 g/L yeast extract, 5 g/L polypeptone, 10 g/L sucrose, pH 7.0–7.2) or on nutrient agar (NA) medium (NB with 15 g/L agar).

Chemical reagents

All chemicals used in these experiments were purchased from commercial suppliers without further modification or purification. Methanol (MeOH, Tedia company, USA), ethanol (EtOH, Sinopharm, China), isopropanol (IPA, Sinopharm, China), acetone (Sinopharm, China), acetonitrile (ACN, Tedia company, USA), ethyl acetate (EA, Sinopharm, China), dimethyl formamide (DMF, Coolaber, China) and dimethyl sulfoxide (DMSO, Coolaber, China) were used in the experiments. Zinc thiazole (98.7% purity) and 20% zinc thiazole suspension concentrate (SC) were provided by Zhejiang Xinnong Chemical Co. Ltd. Adjuvant-98B was purchased from Changzhou Runyuan Pear Products Co.

Mass spectrometry analysis

High resolution electrospray ionization (HR-ESI) mass spectra were obtained on a Sciex Triple Time Of Fly (TOF) 5600 mass spectrometer. TOF-MS spectra were collected in positive mode, with 146 cycles (period cycle time: 800 ms), a pulse frequency of 23.983 kHz and 250 ms accumulation time. To confirm the presence of the desired structure, the peak at 328.8702 Da was analyzed by MS/MS. TOF-MS/MS spectrum of the mentioned peak was carried out in positive mode, with 146 cycles (period cycle time: 800 ms), a pulse frequency of 23.983 kHz and 100 ms accumulation time.

Antimicrobial activity of zinc thiazole against *Xoo*

The antibacterial activity of zinc thiazol against *Xoo* strain PXO99 was examined in "90 mm × 15 mm" Petri dishes (JingAn, China). *Xoo* strain PXO99 was grown in 50 mL NB medium at 28 °C with shaking at 200 rpm. One milliliter of the culture medium at the early logarithmic phase (OD_{600} = 1.0) was centrifuged at 8000×g and 4 °C. The upper phase was discarded, and the harvested cells were suspended in an equal volume of sterilized ddH_2O. Then, 300 μL bacterial solution pre-mixed with 30 mL NA was poured in each plate. After solidification, five holes were drilled in the NA dish using a small hollow aseptic steel pipe. Finally, zinc thiazole suspension concentrate (200 μg/μL) and DMSO-solved zinc thiazol (20 μg/μL) were added into four holes (30 μL per hole) on the dish. Thirty microliters DMSO solvent (without dilution) was added in the middle hole as a control. The plate was placed at 28 °C without shaking, and the bacteriostatic diameters were measured after 48 h cultivation. Each experiment was performed three times with 6 replicates each time.

Half maximal effective concentration (EC_{50})

Xoo, *Xcc*, *Xag*, *Ep* and *Ea* were grown in 30 mL NB medium at 28 °C with shaking at 200 rpm. One milliliter of the bacterial culture at the early logarithmic phase (OD_{600} = 1.0) was centrifuged at 8000×g and 4 °C. After discarding the supernatant, cells were suspended in an equal volume of sterilized ddH_2O. Then, 10 μL bacterial cell suspension was added to 990 μL of fresh NB medium containing zinc thiazol at different concentrations (0, 5, 10, 20, 30, 40 and 50 μg/mL). Zinc thiazol was added in DMSO solution (2.5 μL). The same volume of DMSO, in the absence of zinc thiazole, was used as a negative control. All cultures were shaken at 200 rpm and 28 °C in the dark. The OD_{600} value of the bacterial culture was measured at 24 h. Each experiment was performed three times, with 3 replicates each time.

Measurement of the effect of zinc thiazole on bacterial growth

Xoo strain PXO99 was incubated with shaking at 200 rpm in NB medium (30 mL) at 28 °C until OD_{600} = 1.0 (early logarithmic phase) was reached. One milliliter of the bacterial culture was centrifuged at 8000×g and 4 °C. The upper phase was discarded and the cells were suspended in an equal volume of sterilized ddH_2O. Then, 500 μL of bacterial cell suspension was added to 49.5 mL of fresh NB medium containing zinc thiazole at different concentrations (0, 25 and 50 μg/mL). DMSO-solved zinc thiazole was used in the experiments (125 μL), and DMSO (125 μL) in the absence of zinc thiazole was used as a negative control. Ampicillin at 50 μg/mL concentration was used as a positive control. All cultures were shaken at 200 rpm and 28 °C in dark, and the OD_{600} value was measured every 4 h until control group reached the stationary phase. Each experiment was performed three times, with three replicates per experiment.

Transmission electron microscope (TEM) and scanning electron microscope (SEM) observations

Xoo strain PXO99 was incubated with shaking at 200 rpm in NB medium at 28 °C until OD_{600} = 1.0 (early logarithmic phase) was reached. One millilitre of the culture medium was centrifuged at 8000×g and 4 °C. The cells were suspended in an equal volume of sterilized ddH_2O. Then, 500 μL of bacterial cell suspension was added to 49.5 mL of fresh NB medium containing different zinc thiazol concentrations (0 μg/mL and 25 μg/mL). Zinc thiazol was added as a DMSO solution (62.5 μL), and DMSO (62.5 μL) in the absence of zinc thiazole was used as a negative control. All cultures were grown at 28 °C with shaking at 200 rpm for 12 h. For TEM, bacterial samples were placed on copper mesh grids with formvar membranes and negatively stained with phosphotungstic acid (2% v/v, pH = 6.7). The samples were then observed using a TEM instrument (Hitachi H-7650, Tokyo, Japan) at 80 kV and photographed with a Gatan832 CCD camera (Gatan, Pleasanton, CA, USA).

For SEM, the bacterial cells were washed with 2 mL phosphate-buffered saline. Then, samples were passed through a series of ethanol solutions in ascending order (30%, 50%, 70%, 80% and 100% ethanol) for 10 min per step to remove water. The dehydrated samples were coated with a thin layer of golden (< 5 nm) using an ion sputter and observed using a SEM (EVO-LS10, Carl Zeiss AG, Germany) at different magnifications.

Determination of cell division genes by quantitative real-time PCR

Total RNA was isolated from PXO99 cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and the first strand cDNA was synthesized by using a cDNA Synthesis kit (Takara, Bio, Japan). PCR primers were designed using Primer 5.0 (Additional file 1: Table S1). Next, qRT-PCR was performed using diluted cDNA and SYBR Green PCR Master Mix (Takara, Bio, Japan) on a Quant Studio 6 Real Time PCR system (Thermo Fisher Scientific, Foster, USA). The expression data, given as delta Ct (cycle threshold), were collected and statistically processed using the $2^{-\Delta(\Delta Cp)}$ method. *RecA* was used as an internal control, and each experiment was conducted 3 times with cDNA prepared from different samples. The significant differences were identified by Student's t test.

Evaluation of the efficacy of zinc thiazole in DMSO for the control of rice bacterial blight disease

The efficacy of zinc thiazol was tested using virulent pathogen *Xoo* strain PXO99, which shows stable pathogenicity on *Oryza sativa* subsp. *japonica* var. Nipponbare (susceptible rice). PXO99 was grown in NB medium and shaken at 28 °C and 200 rpm for 16–24 h. Then, the bacterium was suspended in water and the concentration was adjusted to OD_{600} = 1.0. The bacterial inoculation was performed in a greenhouse on the rice leaves (4–5 weeks old) using the leaf clipping method (Kauffman et al. 1973). An aqueous solution (100 mL) containing different concentrations of zinc thiazole (0, 12.5, 25, 50, 100 and 200 μg/mL) was used. One hundred milliliters water containing 1 mL DMSO in the absence of zinc thiazole was used as a negative control. To measure the curative activity, the above 100 mL solutions were hand-sprayed on 5-week-old plant leaves at 12 hpi using PXO99. To screen the protective activity, the above solutions were hand sprayed on plant leaves at 12 h before the inoculation of PXO99. All 100 mL aqueous solutions were sprayed evenly on a 1 m^2 with 15 plants. The lesion length (cm) in leaves was measured after 7 days. Leaves were inoculated with water, in the absence of zinc thiazole, as a negative control. Forty five leaves were inoculated with PXO99 in each treatment. The same experiment was performed three times. Efficacy = (average lesion length in control group - average lesion length in treatment group) / average lesion length in control group × 100%. The variables were analyzed using Student's t-tests and were tested for significance at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) and $P < 0.0001$(****) levels.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s42483-019-0036-4.

Additional file 1: Table S1. Primers used for RT-PCR to detect the mRNA expressions of cell division related genes.

Abbreviations

ACN: Acetonitrile; BB: Rice bacterial blight; BLS: Rice bacterial leaf streak; DMF: Dimethyl formamide; DMSO: Dimethyl sulfoxide; *Ea*: *Erwinia amylovora*; *Ep*: *Erwinia pyrifoliae*; EtOH: Ethanol; IPA: Isopropanol; JA: Jasmonic acid; MeOH: Methanol; NA: Nutrient agar; SA: Salicylic acid; SC: Suspension concentrate; *Xag*: *Xanthomonas axonopodis* pv. *glycines*; *Xcc*: *Xanthomonas campestris* pv. *campestris*; *Xoo*: *Xanthomonas oryzae* pv. *oryzae*

Acknowledgements

We are grateful to Zhejiang Xinnong Chemical Co. Ltd. for providing us the zinc thiazole used in this study.

Authors' contributions

FL and XC designed the study; XC and YZ performed the experiments; XC and KL analyzed the data; XC and PL drafted the manuscript; FL and LZ reviewed and edited the manuscript. All authors read and approved the final manuscript.

Funding

This research was supported by grants from the National Natural Science Foundation of China (31571974), the Special Fund for Agro–scientific Research in the Public Interest (201303015), the National Key R&D Program of China (2017YFD0200900) and the Natural Science Foundation of Jiangsu Province of China (BK20170606).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 21 May 2019 Accepted: 9 September 2019
Published online: 21 October 2019

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