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The biological control of the grapevine downy mildew disease using *Ochrobactrum* sp.

*Chaoqun Zang[1], Qiujun Lin[2,3], Jinhui Xie[1], Ying Lin[1], Kuihua Zhao[1], Chunhao Liang[1]**

[1]Institute of Plant Protection, Liaoning Academy of Agricultural Sciences, Shenyang, P.R. China
[2]College of Plant Protection, Shenyang Agricultural University, Shenyang, P.R. China
[3]Risk Assessment Laboratory for Agricultural Products, Ministry of Agriculture and Rural Affairs, Liaoning Academy of Agricultural Sciences, Shenyang, P.R. China
**Corresponding Author: liangchunhao99@126.com*
Qiujun Lin contributed equally to this work.

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Abstract: Grape downy mildew, caused by *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni 1888, is a widespread fungal disease that causes serious harm to the grape production. The long-term continuous use of chemical pesticides has caused issues with the resistance, residues and resurgence, as well as creating environmental pollution and the declining quality of the products. A biological control offers a safe and effective method to control diseases. We determined the *in vitro* antagonistic activity of 303 bacterial strains from infected grapevine leaves, and 12 isolates showed some level of antagonism in a detached leaf assay. Isolate SY286 reduced the disease severity in the detached leaves by 93.18%, and showed good control effects in a field assay. The scanning electron microscopy showed the damaged *P. viticola* cell walls when the mycelia and sporangia were treated with the fermentation liquor of isolate SY286. Furthermore, it showed an antagonistic activity against *Phytophthora capsici*, *Phytophthora infestans*, *Botrytis cinerea*, *Fusarium oxysporum*, *Colletotrichum orbiculare*, *Trichothecium roseum*, and *Botryosphaeria berengeriana*. The isolate was identified as *Ochrobactrum* sp. combined with its morphological characteristics, physiological and biochemical reactions and 16S rDNA sequence analysis, and it has the potential to control the grapevine downy mildew.

Keywords: *Plasmopara viticola*; antimicrobial activity; biocontrol agent; phyllosphere microorganism

The grapevine (*Vitis vinifera* Linnaeus), cultivated on more than 7.6 million hectares worldwide, is one of the most important economic crops, responsible for the production of 25 billion l of wine in 2017 (OIV 2018). A wide variety of pathogenic microorganisms, such as bacteria, oomycetes and deleterious fungi, can cause diseases that affect the fruits' sensorial and organoleptic properties, resulting in a poor wine quality and major economic losses for the wine producers (Van Helden 2008).

Grapevine downy mildew, caused by *Plasmopara viticola* (Berk. & M. A. Curtis) Berl. & De Toni 1888, is one of the most severe grapevine diseases. Most cultivars of *V. vinifera* are highly susceptible to *P. viticola*, and controlling it requires regular fungicide applications. Most of these fungicides contain copper, which may lead to soil and groundwater pollution (Aziz *et al.* 2006). Recently, synthetic fungicides with specific modes of action have supplemented the copper-based fungicides which have given rise

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to the development of resistant strains of *P. viticola* (Matasci *et al.* 2008). The research results showed that *P. viticola* easily developed resistance to metalaxyl (Sun *et al*. 2010; Bi *et al.* 2014) and azoxystrobin (Wang *et al.* 2018).

As far as we know, biological controls for plant diseases have a lot of advantages, such as being safe for people and animals, being environmentally friendly, and not easily causing the pathogens to develop resistance to chemical pesticides (Peng *et al.* 2015), and the biological control of plant pathogens has emerged as a promising alternative to synthetic pesticides and fungicides. Several phyllospheric microorganisms have been used extensively as biological control agents to control plant diseases (Redmond 1987; Andrews 1991; Nair *et al.* 2002). There are several mechanisms through which biological control agents exert their beneficial effects on plants, including the production of siderophores, hydrogen cyanide (HCN), lytic enzymes, competition, and inducing systemic resistance (Pieterse *et al.* 2001; Tag *et al.* 2003). Several microorganisms, isolated from the rhizosphere or grape fruit surfaces have been selected as biological control agents to control *P. viticola*, such as *Bacillus subtilis* KS1, *Lysobacter capsici* AZ78, *Trichoderma harzianum* T39 and *Fusarium proliferatum* G6 (Falk *et al.* 1996; Perazzolli *et al.* 2008; Furuya *et al.* 2011; Puopolo *et al.* 2014). and *Streptomyces* sp. ANK313, which can generate khatmiamycin, has been confirmed that it can be used for the biological control of *P. viticola* (Abdalla *et al.* 2011). However, only limited attempts have been made to control *P. viticola* infections in the grapevines. The purpose of this study was to evaluate the potential of the phyllospheric microorganisms on *V. vinifera* to control the grapevine downy mildew caused by *P. viticola*. We first isolated the bacteria from the infected plants and then determined their capacity to control the disease and then investigated the potential mechanisms.

MATERIAL AND METHODS

Sample collection and isolation of the bacteria. In July 2011, leaves were collected from healthy grapevines (centennial seedless, 5 years old) in nine different locations (Beizhen, Shenyang, Beijing, Baoding, Tianjin, Hefei, Shanghai, Lanzhou, and Urumqi) from fields infected by *P. viticola*. From each location, ten grapevine leaves were transferred into sterile plastic bags, placed on ice, transported to the laboratory, and processed within 18 to 24 hours. The *bacillus* species were isolated from the leaves according to Narita *et al.* (2004). The isolates were maintained on beef extract-peptone medium slants.

Sporangia suspension preparation of P. viticola. The diseased leaves of the centennial seedless grapes were collected, and then washed with sterile water. The petiole was wrapped with a wet absorbent cotton, and the abaxial surface of the leaves was upturned, and was placed in a culture dish, and then cultured in the incubator at 22°C for 24 hours. The sporangia were collected with a sterile brush, and then the suspension was diluted into 10^5 sporangia per millilitre. This sporangia suspension was prepared for the detached leaf assay.

Detached leaf assay. We used 303 isolates for the detached leaf assay. The grapevine leaves were collected from a two-month-old grapevine of centennial seedless grapes, and the leaf surfaces were sterilised with a 1% sodium hypochlorite solution for 30 s and rinsed several times with sterile water. The isolate concentrations of approximately 10^8 cells per ml were sprayed on the separate detached leaves and placed in Petri plates lined with a moistened filter paper. A sporangium suspension with a concentration of approximately 10^5 sporangia per ml was prepared and sprayed onto the leaves after the isolates. Later, the leaves were incubated in a growth chamber at 22°C with 12 h light/dark for 10 days. The disease severity was assessed using a six-point scale based on the area of the leaves covered in white lesions: 0 (no symptoms); 1 (below 5%); 3 (6 to 25%); 5 (26 to 50%); 7 (51 to 75%); and 9 (more than 75%) (Yu *et al.* 2016). Leaves without the antagonistic bacteria served as a control. Each treatment consisted of three detached leaves, and each treatment was undertaken in three replicates (Chiou & Wu 2001). The control effect was calculated using the disease index (DI)(Equations 1 and 2):

$$\mathrm{DI} = \frac{\sum(A \times B)}{M \times B_{\max} \times 100} \quad (1)$$

where: A – the number of diseased leaves from all the levels; B – the level of each diseased leaf; M – the total number of the leaves; B_{max} – the highest level of the disease.

$$I(\%) = \frac{Z_{ck} - Z_x}{Z_{ck}} \times 100 \quad (2)$$

where: I – the control effect; Z_{ck} – the disease index of control group; Z_x – the disease index of the treatment group.

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Biological control efficiency in the field assay. Based on the results of the detached leaf assay, isolate SY286 was selected to test the biological control efficiency in a field of centennial seedless grapes (table grape variety) from 2016 to 2017. The isolate was incubated in a shaking incubator with a beef extract-peptone liquid medium for 72 h at 27°C, and the concentration was approximately 10^8 cells per ml. It was named "the original fermentation solution", and it was used as one treatment. The original fermentation solution was diluted 200 and 400 times for another two additional treatments. The fungicides Equation pro (containing 22.5% famoxadone and 30% cymoxanil) and metalaxyl mancozeb (containing 10% metalaxyl and 48% mancozeb) were used as the chemical agent control, the Equation pro and metalaxyl mancozeb fungicides are frequently-used agents for controlling grapevine downy mildew in production. Both the Equation pro and metalaxyl mancozeb are compound fungicides, and they were combined with a protective fungicide and a systemic fungicide. Fresh water was used as another control. The water consumption was 1250 l/ha. There were three treatments and three control-treatments, and each treatment had four replicates. Therefore, there were twenty-four test plots in a random order (Figure 1). Each test plot was about 50 m^2. Most of the information about the treatment date and the precipitation is listed in Table 1.

The grapevines in the treatment test plots were sprayed with the biological control agent and the fungicides separately once every seven days one time each, and it was done four times in total. The grapevine plants were first sprayed with the biological control agent or the fungicides before scabs were found. Before the third spray, the medium-term control effect was surveyed. The final control effect was surveyed 10 days after the last spray. One branch was randomly selected on each grapevine, and we determined the disease indices of 15 leaves from the top leaf to the fifteenth leaf. The disease indices and control effects were calculated using the same formula described earlier.

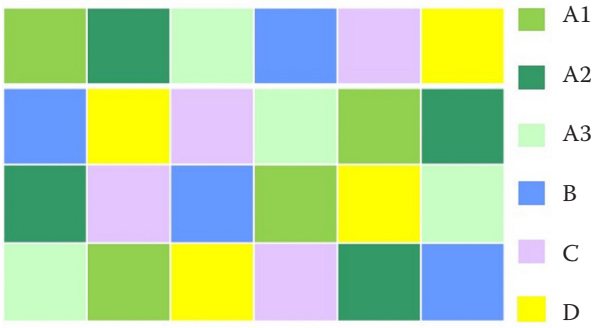

Figure 1. The layout of the biological control efficiency in the field assay

A1 – the original fluid of isolate SY286; A2 – the A1 dilutes 200×; A3 – the A1 dilutes 400×; B – 2000 times of equation pro; C – 1000 times of metalaxyl mancozeb; D – the control treatment of clear water

Evaluation of the effectiveness of isolate SY286 based on the 16S rDNA homology. Based on the results of the detached leaf assay and field assay, isolate SY286 showed a strong inhibitory activity against *P. viticola*. Identification of the isolate SY286 was based on the 16S rDNA sequence. To extract the DNA, the cells were harvested from 10 ml of an overnight incubated culture, and the pellets were lysed in a 1 ml lysis buffer (25% sucrose, 20 mM EDTA, 50 mM Tris-HCl and 5 mg/ml of lysozyme).

Table 1. The weather conditions on the day of the application

Date	Temperature (°C)			Rainfall (mm)	Avg. wind velocity (m/s)	Avg. relative humidity (%)
	max.	min.	avg.			
2016						
June 20	30.0	16.1	23.7	0	1.9	71.0
June 27	28.6	17.7	23.4	0	1.5	69
July 4	32.4	19.3	26.2	0	0.9	69
July 11	33.7	21.9	29.2	0	2.9	63
2017						
June 22	27.8	17.1	23.0	0	1.4	67
June 29	31.3	20.0	26.1	0	3.2	64
July 6	36.4	20.7	29.3	0	2.0	58
July 13	33.1	24.3	28.9	0	3.1	75

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The chromosomal DNA was extracted according to Sambrook *et al.* (1990).

The 16S rDNA was amplified by polymerase chain reaction amplification (PCR) with the universal primers 16f (AGAGTTTGATCCTGGCTCAGAACGAACGT) and 16r (TACGGCTACCTTGTTACGACTTCACCCC). The PCR reaction system was composed of 3.0 µl DNA, 5.0 µl 10× PCR buffer, 2.0 µl upstream primer (16f), 2.0 µl downstream prime (16r), 0.3 µl dNTP, 0.3 µl taq polymerase, 37.4 µl double-distilled H_2O. The amplification conditions were setup for the initial denaturation at 94°C (5 min), followed by 35 cycles at 94°C (1 min), 49°C (2 min) and 72°C (2 min), with a final extension at 72°C for 7 minutes. The PCR products were sequenced by Shanghai Sangon Biotech, China. The sequences were compared using the Mega program (version MEGA7.0) to identify the isolates.

Classification status of isolate SY286 based on the morphological characteristics and the physiological and biochemical reactions. The colonial morphology of the bacterial isolate SY286 was observed on a beef extract-peptone medium by direct and optical microscope observations of the single colonies. Gram staining was performed according to the procedure described by Claus (1992). The physiological and biochemical reactions of strain SY286 were tested according to Dong (2001). The analysed features include the gelatin liquefaction, the esculin hydrolysis, the oxidase and the contact enzyme, assimilation of the carbon and nitrogen sources, the growth range of the temperature, the pH, and the concentration of NaCl, as well as several physiological and biochemical reactions (Table 2).

Antimicrobial spectrum assay. Isolate SY286 was selected in order to test its antagonistic activity against other fungal plant pathogens, such as *P. capsici*, *P. infestans*, *B. cinerea*, *F. oxysporum*, *C. orbiculare*, *T. roseum*, and *B. berengeriana*, on the Potato Dextrose Agar (PDA) plates using the dual culture technique. The pathogen was put on one side of the plate with the culture medium, and the biocontrol agents were put on the other side, and the treatment only had the pathogen on the plates as the control. The plate was placed in an incubator to culture. After incubation for 5 to 7 days at 25°C, it was observed whether the biocontrol agents could inhibit the pathogen or not (Yoshida *et al.* 2001).

Cellulase, siderophores, HCN and protease production assay. The cellulase production was according to the methods of Ghose (1987). The isolate was inoculated on a cellulose-decomposing-microorganism medium, incubated at 28°C for 3 days, then dyed for 1 h with Congo red (1 mg/ml), and the dye liquor was poured out. It was soaked again for 1h with the dye liquor (1M) and observed for the presence of a transparent zone.

Table 2. The physiological and biochemical characteristics of the SY286 strain

Physiological and biochemical indexes	Result
Salt tolerance	≤ 5%
Growth temperature	5–45°C
Oxidase test	+
Contact enzyme reaction	+
Glucose oxidation fermentation	–
Methyl red test	–
Phenylalanine dehydrogenase test	–
Voges-Prokauer reaction	–
Starch hydrolysis test	+
Gelatin liquefaction test	–
Esculin hydrolysis test	–
Nitrate reduction test	+
Hydrogen sulfide generation test	–
Indole test	–

(+) – positive reaction; (–) – negative reaction

The siderophore secretion was detected by the universal method of Schwyn and Neilands (1987). According to this method, 0.5 ml of a blue Chrome azurol solution was added to 0.5 ml of the filtered supernatant of the isolate. A reference solution was prepared using the uninoculated medium. A positive reaction was estimated by the colour change in the assay reagent from blue to orange. The assay was considered to be negative when there was no colour change within 3 hours.

The HCN production was tested according to the method of Lee *et al.* (2001). The isolate was grown on a tryptic soy agar medium with 4.4 g/l of glycine and filter paper strips soaked in a picric acid solution (2.5 g of picric acid, 12.5 g of Na_2CO_3 in 1 l of distilled water) in the lid of each Petri dish. The dishes were sealed with parafilm and incubated at 28°C for 3 days. The change in the filter paper colour from yellow to brown indicated that HCN was produced by the isolate (Lee *et al.* 2001).

The protease activity of the isolate was determined according to Wei *et al.* (2004). The isolate was puncture-inoculated on a skim milk medium (Reimmann *et al.* 1997). The isolates were cultured at 25°C in an incubator for 3 days. The presence of a transparent zone proved the protease production.

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The effects of the antimicrobial substances from strain SY286 on the ultrastructure of P. viticol. The effect of the antimicrobial substances produced by isolate SY286 on the ultrastructure of the treated and untreated (control) mycelium and sporangium of *P. viticola* was observed using scanning electron microscopy (SEM). The mycelium and sporangium were treated with a fermentation liquor of isolate SY286 for 12 h, and transferred to a glass cover slip, then fixed with 2.5% glutaraldehyde for 12 h at 4°C. These were then dehydrated with a graded series of ethanol washes followed by drying in a desiccator (Walter & Crawford 1995). All the samples were affixed to the SEM stubs using a carbon tape followed by a thin coating of gold and were detected by SEM (Hitachi, Ltd. S4800, Japan).

Statistical analysis. There were three replicates for each treatment of the detached leaf test. The plants for the field assay of the biological control efficiency were arranged in a randomised block design with four replicates in each treatment. The data were subjected to an analysis using one-way ANOVAs, followed by Tukey's multiple means comparisons at $P < 0.05$ (SPSS, version 16.0)

RESULTS

We isolated 303 morphologically distinct bacteria from the phyllosphere of the grapevines, and 12 bacterial isolates showed varying levels of antagonism in the detached leaf assay. Isolate SY286 had the highest control effect (93.18%) (Figure 2), and isolate BZ85 had the lowest (34.67%) (Table 3). In the field test, isolate SY286 exhibited a strong control effect (Table 4), and it also exhibited an antagonistic activity against *P. capsici*, *P. infestans*, *B. cinerea*, *F. oxysporum*, *C. orbiculare*, *T. roseum* and *B. berengeriana* (Table 5).

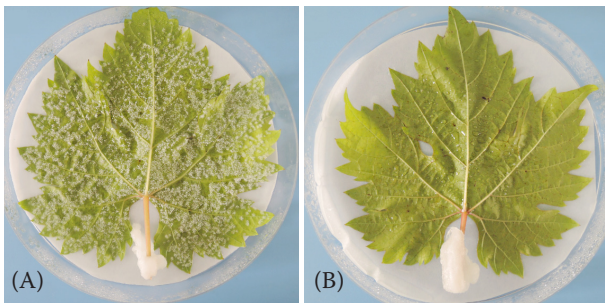

Figure 2. The control effect of the SY286 strain against *Plasmopara viticola* in the *in vitro* condition: (A) the control group and (B) the treatment of the SY286 strain

Table 3. The control effect of the isolates from the grape leaves against *Plasmopara viticola* in the *in vitro* condition

Isolates	Avg. disease index	Control effect (%)
BZ22	55.32	40.25^{f}
BZ24	53.11	42.64^{f}
BZ25	36.90	60.15^{c}
BZ81	60.49	34.67^{g}
BZ85	43.21	53.33^{de}
BZ86	45.68	50.66^{e}
SY224	35.80	61.33^{c}
SY226	29.10	68.57^{b}
SY257	45.68	50.66^{e}
SY266	41.15	55.56^{d}
SY286	6.31	93.18^{a}
SY296	46.00	50.32^{e}
CK	92.59	0

The means followed by the different letters – significantly different ($P < 0.05$) according to the Student-Newman-Keuls test; CK – the control that uses distilled water to replace the isolate suspension liquid

This suggests that the biological control agent SY286 has broad-spectrum antibacterial functions.

The assay for the cellulase production showed a transparent zone surrounding isolate SY286, indicating that the cellulase was one of the metabolites that the organism secreted during the growth. Isolate SY286 produced siderophores during the growth (Figure 3). However, the HCN assay result was negative with no colour change, indicating that isolate SY286 does not produce HCN.

Table 4. The control effect of *Plasmopara viticola* to the grapevine downy mildew on the different treatment in the field

Treatment	Medium–term control effect (%)		Final control effect (%)	
	2016	2017	2016	2017
A1^{a}	83.28^{a}	85.22^{a}	74.51^{b}	79.12^{a}
A2	75.65^{b}	71.99^{d}	70.83^{c}	68.75^{c}
A3	62.19^{c}	58.34^{e}	60.04^{d}	54.22^{d}
B	86.6^{a}	81.88^{b}	85.53^{a}	80.45^{a}
C	73.48^{b}	76.03^{c}	71.08^{c}	72.31^{b}
D	—	—	—	—

A_1 – the original fluid of isolate SY286; A2 – A1 dilutes 200 ×; A3 – A1 dilutes 400 ×; B – 1 : 2000 × of Equation pro for the dilutions; C – 1:1000× of metalaxyl mancozeb for the dilutions; D – the controlling treatment of clear water. The means followed by the different letters – significantly different ($P < 0.05$) according to the Student-Newman-Keuls test

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Table 5. The inhibition of other pathogen mycelial growth by SY286

Isolate	Degrees of inhibition						
	P. capsici	*P. infestans*	*B. cinerea*	*F. oxysporum*	*C. orbiculare*	*T. roseum*	*B. berengeriana*
SY286	+++[a]	+++	+++	++	++	+++	+++

(+) – 1–5 mm wide zone; (++) – 6–10 mm wide zone; (+++) – more than 10 mm wide zone. The results – average of three replicates

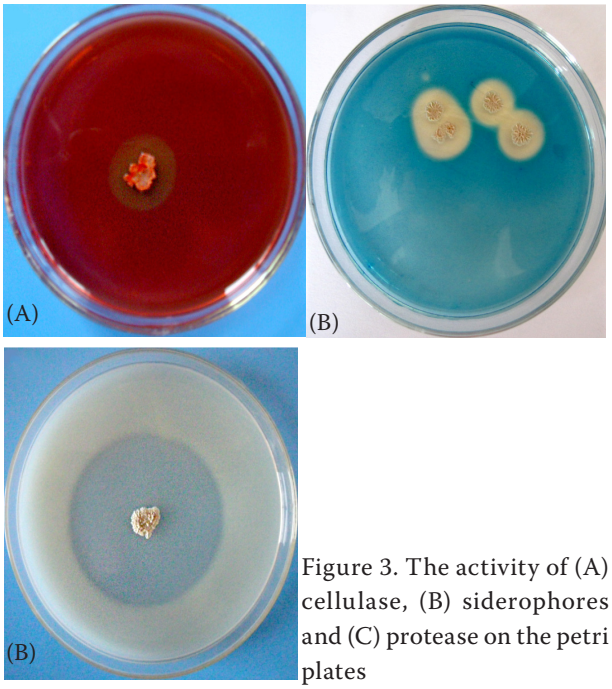

Figure 3. The activity of (A) cellulase, (B) siderophores and (C) protease on the petri plates

The colony of isolate SY286 was colourless, flat, and had a smooth surface. The thallus was bacilliform and gram-negative. It could use various amino acids, organic acids as carbon sources, and it could also use nitrate and ammonium nitrogen as nitrogen sources. Its growth temperature was 5–45°C, and its suitable pH was 6–9. When the NaCl concentration was below 5%, it had normal growth. The catalase reaction, oxidase reaction, amylohydrolysis test and nitrate reduction test were positive, and the gelatin liquefaction test, esculin hydrolysis test, glucose oxidation fermentation, methyl red test, phenylalanine dehydrogenase test, Voges-Prokauer reaction, hydrogen sulfide generation test and indole test were negative (Table 4). The PCR amplification of the targeted 16S rDNA resulted in the predicted 1.4 kbp amplicons in the SY286 isolate. The PCR amplified products were sequenced and compared with the 16S rDNA sequences in BLAST. Isolate SY286 was identified as *Ochrobactrum* sp. combined with its morphological characteristics, physiological and biochemical reactions and 16S rDNA sequence analysis. The 16S rDNA sequence of isolate SY286 was deposited in the GenBank (KC978888).

After 12 h, the effects of the fermentation liquor from isolate SY286 on the morphology of the *P. viticola* mycelia and sporangium were detected by SEM. No morphological changes were observed in the control group (Figure 4A); however, some changes were observed in the morphology of *P. viticola* when treated with the fermentation liquor of SY286. Irregular

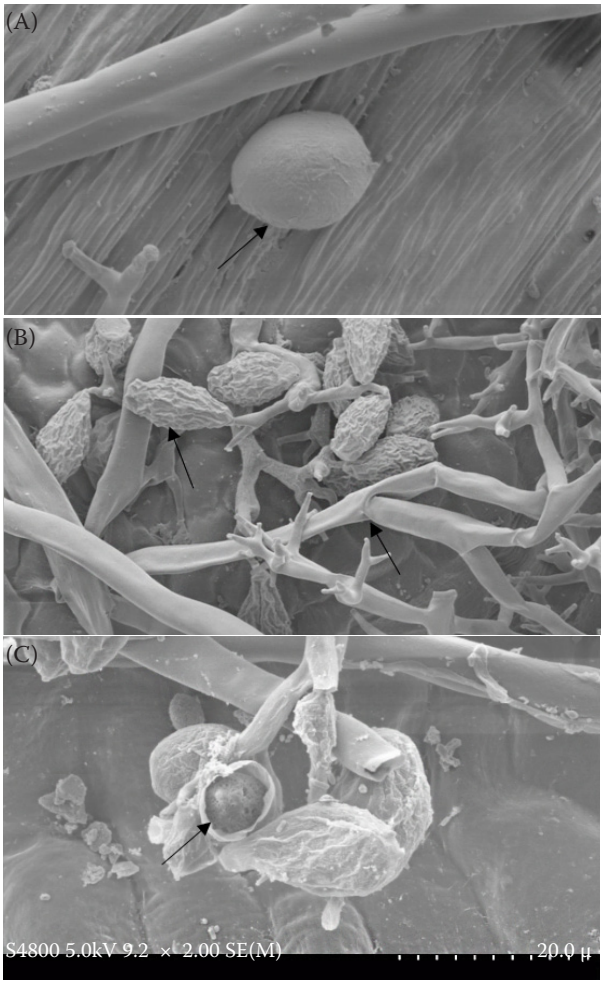

Figure 4. The effect of the fermentation liquor of strain SY286 to *Plasmopara viticola*

The scanning electronic micrographs (SEM) of *Plasmopara viticola* growing on the grape leaves (A) in the absence (control) or (B,C) presence of the fermentation liquor of isolate SY286 after incubation at 22°C for 12 h; arrows – the *P. viticola* mycelia and sporangium damaged by some antibacterial substances in the fermentation liquor of SY286

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distortions were observed in the pathogen's mycelia (Figure 4B), and its sporangia were crimpled or ruptured (Figures 4B and C). These results indicate that there were some substances with antibacterial properties in the fermentation liquor of isolate SY286, and they played a critical role in disrupting the mycelia and sporangium of *P. viticola*.

DISCUSSION

A strain called SY286 was isolated from the phyllosphere of the grapevine leaves infected with grapevine downy mildew, and it showed a promising inhibitory activity against *P. viticola* in both the *in vitro* and in planta. There are abundant microorganisms that exist on the plants that can be used as biological control agents. Phyllospheric microorganisms were the first mentioned by Blackman (1981). Like rhizospheric microorganisms, they also have antagonism against pathogens. *Bacillus amyloliquefaciens* is a common antagonistic bacteria and *Bacillus amyloliquefaciens* BS6, isolated from the phyllosphere, has been used to prevent *Sclerotinia sclerotiorum* (Lib.) de Bary efficiently in the field (Fernando *et al.* 2007). A mass of *Bacillus thuringiensis* Berliner, 1915 colonised on the phyllosphere of a platyphyllous garden sorrel, reaching a concentration of 1.9×10^4 per gram (Fay *et al.* 2005), could protect plants from herbivorous insects (Smith *et al.* 1991).

Our results indicate that the metabolites of isolate SY286 included cellulase, siderophores, and protease (Figure 3). The results of SEM also demonstrated that strain SY286 could destroy the mycelia and sporangium of *P. viticola.* The cellulase, siderophores, and protease are often the metabolites of biological control agents against pathogens. The *Trichoderma* species have been reported as biological agents that produce cellulolytic enzymes against *Fusarium moniliforme* Sheldon and *Aspergillus flavus* Link, 1809 (Claudia *et al.* 1997). Some siderophore-producing rhizobacteria suppress some soil-borne fungal pathogens (Gupta *et al.* 2002). There has been convincing evidence supporting the direct function of the siderophore-mediated iron competition as a biological control ability. Siderophore-producing strains rapidly colonise the plant roots of some crops, increasing the yields (Hass & Defago 2005). Siderophore-producing strains have been screened as potential biological control agents for rice fungal diseases in Thailand (Mathurot *et al.* 2009). Protease has also been shown to be an important mechanism in the biological control. *Isaria fumosorosea* (Wize) could produce cuticle degrading enzymes, including protease, as a biological control agent against the diamondback moth (Shaukat *et al.* 2010). *Phytophthora capsici* Leonian, 1922, oomycetes, was one damaging pathogen that could cause pepper phytophthora blight. This soil-borne disease could cause serious harm to chili production. The main components of the cell walls of *P. capsici* is cellulose. The antimicrobial spectrum test showed that strain SY286 had a strong inhibitory activity against *P. capsici*. Strain SY286 could secrete cellulase and siderophores, the cellulase could destroy the cell wall of the pathogen, and the siderophores could compete with the pathogen for the necessary iron ion. It might have a broad prospect on controlling pepper phytophthora blight.

Isolate SY286 was identified as *Ochrobactrum* sp. The *Ochrobactrum* strains exist in various habitats, including plants, the rhizosphere, animals, water, and humans. *Ochrobactrum* spp. are often associated with plants because they have frequently been isolated from the rhizoplane or the rhizosphere (Lebuhn *et al.* 2000). *Ochrobactrum*, which is believed to be able to fix nitrogen (Magalhães *et al.* 2001), was recently proposed as having symbiotic relationships with *Acacia* and *Lupinus* nodules (Ngom *et al.* 2004; Trujillo *et al.* 2005). Recent reports have described strains of *Ochrobactrum* in the plant tissue of deepwater rice, as well as from soils and sediments (Tripathi *et al.* 2006). These strains were associated with the detoxification of xenobiotics, especially in halobenzoates under denitrifying conditions (Song *et al.* 2000). Furthermore, *O. anthropi* produced several kinds of enzymes containing D-transferase aminopeptidase and glutathione S-transferase that could utilise atrazine as the sole source of carbon (Fanuel *et al.* 1999; Favoloro *et al.* 2000). The biocontrol potential of the phylloplane bacterium *Ochrobactrum anthropi* BMO-111 showed an obvious controlling effect on the blister blight disease of tea (Sowndhararajan *et al.* 2013).

Nowadays, there have not been any reports about *Ochrobactrum* sp. being used to control the grapevine downy mildew disease. The isolate *Ochrobactrum* sp. SY286 had a good inhibitory activity against *P. viticola* in the detached leaf assay and in the field assay, and it could secrete some active substances, such as cellulase, siderophores, and protease. Furthermore, we also studied the structure of the bioactive compounds and their antimicrobial mechanism.

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As far as we know, biological controls can become one of the potential ways to control plant diseases. The SY286 strain was isolated from the healthy grapevine leaves, and it showed a strong inhibitory activity against *P. viticola* and other important pathogens. It has broad application prospects for the protection against grapevine downy mildew and other plant diseases. This study has demonstrated the inhibition of strain SY286 against *P. viticola* using a detached leaf assay in the field, and has confirmed that the fermentation liquor of the SY286 strain could cause the sporangia and sporangiophore to be crimpled or ruptured. Furthermore, we have preliminarily analysed a few active substances in the fermentation liquor of the SY286 strain, suggesting the development of a biological control agent for the grapevine downy mildew.

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