Effects of *Serratia marcescens* (SM1) and its interaction with common biocontrol agents on the termite, *Odontotermes formosanus* (Shiraki)

Renjie Fu^{1,2} · Luxiang Zhou^{1,2} · Kai Feng^{1,2} · Xiaoyu Lu^{1,2} · Jian Luo^{1,2} · Fang Tang^{1,2}

Received: 4 November 2019 / Accepted: 19 December 2019 / Published online: 30 June 2020
© The Author(s) 2020

Abstract *Odontotermes formosanus* (Shiraki), a black-winged subterranean termite, is a common forest pest. A red pigment-producing bacterial strain isolated from the termite was identified as *Serratia marcescens* and named SM1. A bioassay of SM1 on *O. formosanus* show that the LD₅₀ ranged from 1.77×10^4 to 10.82×10^4 cells/termite over 21–39 h. Three biological control agents, *Beauveria bassiana* (2×10^{10} cells/mL), *Metarhizium anisopliae* (1×10^{10} cells/mL) and *Bacillus thuringiensis* (1.6×10^8 IU/mL), were used for an *O. formosanus* bioassay. The results show that the insecticidal effect of *B. bassiana* was stronger than that of *M. anisopliae*. In addition, two mixtures were obtained by combining *B. bassiana* (2×10^{10} cells/mL) with SM1 (1.5×10^{10} cells/mL), and *M. anisopliae* (1×10^{10} cells/mL) with *S. marcescens* (SM1) (1.5×10^{10} cells/mL) in equal volumes. The results show that *B. bassiana* and SM1 was less effective than SM1 alone. However, the insecticidal effect of

M. anisopliae and SM1 was better than that of *M. anisopliae* or SM1 individually. These studies provide an important contribution for termite biocontrol.

Keywords *Odontotermes formosanus* (Shiraki) · *Serratia marcescens* strain SM1 · LD₅₀ · Biological control agents

Introduction

Current control methods for *Odontotermes formosanus* (Shiraki) rely mainly on chemical treatments, including spraying liquid chemicals, feeding bait, dusting and grouting. The active ingredients in these chemicals are synthetic organic pesticides that present different levels of threat to soil, water and the human body. Therefore, alternative, environmentally friendly termite control agents such as biological control agents need to be developed (Liang et al. 2012). An active bacteria strain isolated from *O. formosanus*, was identified as *Serratia marcescens* (SM1) (Fu et al. 2019).

S. marcescens, is a Gram-negative bacterium and an important member of the *Enterobacteriaceae*. It can effectively control a number of pests and pathogenic bacteria in the field of biological control (Babashpour et al. 2012; Wang et al. 2013) and can also enhance plant resistance to pathogenic bacteria (Nobutaka et al. 2005; Lavania et al. 2006; Chakraborty et al. 2010; Ting et al. 2010). In recent years, it has been found that *S. marcescens* has insecticidal properties. Liu et al. (1988) found it in diseased planthoppers. Wang et al. (2010) isolated a strain of *S. marcescens*, KI3, which showed an obvious toxic effect to aphids. In addition, a strain of *S. marcescens* isolated from the cotton bollworm, *Helicoverpa armigera* Hübner, had a strong pathogenicity and control on *H. armigera*, on the cabbage white worm, *Pieris rapae* L. and beet armyworm,

Project funding: The work was supported by the Zhenjiang's Key R & D Program-Modern Agriculture (NY201702), Jurong Agricultural Science and Technology support plan (NY2017846771), the Priority Academic Program Development Fund of Jiangsu Higher Education Institutions, and the Science and Technology Innovation of Nanjing Forestry University students.

The online version is available at <http://www.springerlink.com>

Corresponding editor: Tao Xu.

✉ Fang Tang
tangfang76@sohu.com; tangfang76@foxmail.com

¹ Co-Innovation Center for the Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, People's Republic of China

² College of Forestry, Nanjing Forestry University, Nanjing 210037, People's Republic of China

Spodoptera exigua (Hübner) (Chen et al. 2001). The development and utilization of *S. marcescens* bacterial strains and their mechanism of action on plant pests has attracted increasing attention from researchers worldwide. However, the application of *S. marcescens* to *O. formosanus* has not yet been studied.

Mixing pesticides may increase their toxicity, and the combined use of different bacteria may also increase the preventive effects of pesticide treatment. Yin et al. (2004) found that chitinase enzymes produced by *S. marcescens* hydrolyze chitin in the midgut peritrophic membranes of locusts and perforate intestinal membranes, which makes insecticidal proteins of *S. marcescens* more likely to penetrate into the insect body and destroy the digestive tract, thereby greatly improving the insecticidal effect on the locusts. At the same time, chitinases produced by *S. marcescens* also cause significant damage to allow other pathogens to invade locusts and increase the infection and fatality rates. Chitinases that is beneficial for Bt infection have a synergistic effect on *Bacillus thuringiensis* (Bt) (Sampson and Gooday 1998). In addition, Liu et al. (1988) reported a synergizing effect from the mixed use of *S. marcescens* and *B. thuringiensis* strain HD-1. However, the toxicity of *S. marcescens* to *O. formosanus* has not been reported. Therefore, the SM1 strain of *S. marcescens*, which we isolated and identified, was used to (1) determine the degree of toxicity to *O. formosanus*; and, (2) determine whether the toxicity of the mixture increased after *S. marcescens* was mixed with other biological control agents.

Materials and methods

Isolation and culture of the *S. marcescens* strain, SM1

S. marcescens strain SM1 was isolated from infected *O. formosanus* and was deposited at Nanjing Forestry University in Nanjing, China. Solid bacterial medium (1 L) consists of peptone 10 g, beef extract 20 g, NaCl 2 g, K₂HPO₄ 2 g, agar 18 g and H₂O 1 L, pH 7.2–7.4.; Seed medium (1 L) consists of peptone 10 g, yeast extract 20 g, NaCl 2 g, K₂HPO₄ 2 g and H₂O 1 L; Zymotic medium (1 L) consists of peptone 10 g, soybean oil 30 g, NaCl 2 g, K₂HPO₄ 2 g and H₂O 1 L. The isolated SM1 strain was cultured on solid media without light at 27 °C. A single colony was isolated, placed in a 250 mL Erlenmeyer flask containing 50 mL sterilized seed medium and incubated for 12 h at 30 °C and 200 r/min. The 70 mL seed solution was added to 250 mL zymotic medium and cultured in a shaking incubator at a rotational speed of 200 r/min at 30 °C for 36 h (Zhang et al. 2015).

Insects

Eight colonies of *O. formosanus* were collected from Jurong County in Zhenjiang, Jiangsu Province and kept in sealed plastic containers in total darkness at 27 ± 1 °C and $75 \pm 1\%$ relative humidity. All colonies were maintained under laboratory conditions without soil and with moist filter paper for 1 day before treatments.

Determination and dilution of the concentration of SM1

The zymotic medium was used as stock liquid and diluted and quantified. A drop of the bacterial liquid was used to fill the counting area made up of 25 squares, and the number of bacteria in five squares (upper left, lower left, upper right, lower right and centre) was counted according to their diagonal position.

Calculation formula:

$$\text{SM1 cells/L} = \text{number of bacteria in the five squares} / (5 \times 25 \times 10 \times 10^6 \times \text{dilution factor})$$

where, (The number of bacteria in the five squares)/5 is the average number of bacteria in the five middle squares (pink), and $N/5 \times 25$ the total number of bacteria in the center (i.e., the total number of bacteria in 0.1 mm^3). $N/5 \times 25 \times 10$ is the total number of bacteria in 1 mm^3 , and $N/5 \times 25 \times 10 \times 10^6$ the total number of bacteria in 1 L.

The concentration of the stock liquor was 1.5×10^{10} cells/mL, diluted to 10 , 10^2 , 10^3 and 10^4 to obtain 1.5×10^9 , 1.5×10^8 , 1.5×10^7 and 1.5×10^6 cells/mL.

Source and concentration of three biological agents

Beauveria bassiana and *Metarhizium anisopliae* were obtained from Yancheng Shenwei Microbiological Strain Technology Co. Ltd., and *B. thuringiensis* from Hubei Kangxin Agricultural Pharmaceutical Co., Ltd. The three were diluted as suspensions so that *B. bassiana* was 2×10^{10} cells/mL, *M. anisopliae* 1×10^{10} cells/mL and *B. thuringiensis* 1.6×10^8 IU/mL.

Bioassay of *O. formosanus* with SM1 and three biological agents

Ten *O. formosanus* workers of similar size were placed in a 7-cm petri dish on a wet filter paper. A $0.12 \mu\text{L}$ quantity of the different concentrations of biocontrol agents was titrated on the pronotum of each worker; $0.12 \mu\text{L}$ water was titrated as the control. Each experiment was repeated three times and mortality was observed and recorded every hour in the dark at 27 °C. When the mortality rate of the

control group was approximately 20%, the experiment was concluded.

To study the combination of the two biological agents and SM1, another ten *O. formosanus* workers of similar size were placed in a 7-cm petri dish, on wet filter paper, and *B. bassiana* (2×10^{10} cells/mL) or *M. anisopliae* (1×10^{10} cells/mL) mixed with SM1 (1.5×10^{10} cells/mL) (volume ratio = 1:1). The same method was used for the bioassay experiments. Each experiment was repeated three times and mortality recorded hourly in the dark at 27 °C. When the mortality rate of the control group was approximately 20%, the experiment ended.

Statistical analysis

Data were processed by DPS 4.5 software and LD₅₀ with a 95% confidence interval was obtained. The data were subjected to analysis of variance using InStat software (Graph-Pad, San Diego, CA) with significance defined as $P < 0.05$.

Results

Determination of SM1 toxicity to *O. formosanus*

The bioassay results show that SM1 clearly affected *O. formosanus*, and the higher the concentration, the greater the toxicity. In addition, the longer the processing time of SM1, the stronger the toxic effect. The LD₅₀ of SM1 at 24 h was 6.66×10^4 cells/termite, and at 39 h 1.77×10^4 cells/termite (Table 1).

Toxicity of *B. bassiana*, *M. anisopliae* and *B. thuringiensis* to *O. formosanus*

Comparing the titration results for these three biological agents, toxicity levels of the *B. bassiana* (2×10^{10} cells/mL) and *M. anisopliae* (1×10^{10} cells/mL) were significantly different from the control. *B. bassiana* and *M. anisopliae* were more toxic to *O. formosanus*; *B. thuringiensis* (1.6×10^8 IU/

Table 2 Bioassay of three biological agents on *O. formosanus*

Time (h)	Mortality rate (%)			
	Control group	<i>B. bassiana</i>	<i>M. anisopliae</i>	<i>B. thuringiensis</i>
12	0.0±0.0 b	23.3±5.8 a	0.0±0.0 b	0.0±0.0 b
24	3.3±2.9 b	23.3±5.8 a	16.7±5.8 a	0.0±0.0 b
36	8.3±2.9 ab	26.7±11.6 a	16.7±5.8 ab	6.7±5.8 b
48	8.3±2.9 c	46.7±5.8 a	26.7±11.6 b	10.0±0.0 bc
60	13.3±2.9 b	56.7±5.8 a	26.7±11.6 b	23.3±5.8 b
72	21.7±2.9 b	80.0±0.0 a	36.7±20.8 b	23.3±5.8 b
84	21.7±2.9 c	80.0±0.0 a	50.0±10.0 b	30.0±0.0 c
96	21.7±2.9 b	86.7±5.8 a	76.8±5.8 a	30.0±0.0 b

The average fatality rates (%) of *B. bassiana* (2×10^{10} cells/mL), *M. anisopliae* (1×10^{10} cells/mL) and *B. thuringiensis* (1.6×10^8 IU/mL) to *O. formosanus* were compared using sterile water as the control. Means ± SD within rows and followed by different letters are significantly different (Tukey's test, $P < 0.05$)

mL) was not different from the controls, i.e., there was no toxicity (Table 2).

Comparing the effects of *B. bassiana* and *M. anisopliae* over 48 to 84 h, the mortality rate with *B. bassiana* reached 50% about 48 h, while it required 84 h for the mortality rate with *M. anisopliae* to reach 50%. *B. bassiana* needed approximately 72 h and *M. anisopliae* approximately 96 h to achieve an 80% mortality rate. Therefore, *B. bassiana* had a stronger insecticidal effect on *O. formosanus* than *M. anisopliae* (Table 2).

Toxicity of combined biological agents to *O. formosanus*

The results of the bioassay of the combined biological agents showed that the mixture of *B. bassiana* (2×10^{10} cells/mL) and SM1 (1.5×10^{10} cells/mL) was less effective than using SM1 alone. The effect of the mixture of *M. anisopliae* (1×10^{10} cells/mL) and SM1 (1.5×10^{10} cells/mL) on *O. formosanus* was better than the components used separately. It required 30 h to kill 80% of the *O. formosanus* using only SM1, but only 26 h with the mixture of SM1 and *M. anisopliae* (Table 3).

Table 1 Toxicity of *S. marcescens* strain SM1 to *O. formosanus*

Time (h)	Toxicity regression line	LD ₅₀ (95% confidence interval) ($\times 10^4$ cells/termite)
21	$y = 4.0066 + 0.9607x$	10.82 (6.04–19.35)
24	$y = 3.9949 + 1.2203x$	6.66 (4.23–10.49)
27	$y = 4.3135 + 1.3613x$	3.19 (1.64–6.20)
29	$y = 4.3081 + 1.3940x$	3.14 (1.62–6.07)
39	$y = 4.7059 + 1.1914x$	1.77 (0.58–5.39)

Discussion

Control of *O. formosanus* is mainly through chemical means and the effective components of pesticides are synthetic organic compounds. These compounds present different degrees of toxicity levels to soils, water and to humans. Although development of the practical application of biological controls is progressing slowly, its environmental and

Table 3 Bioassays of the combination of two biological agents and *S. marcescens* strain SM1 on *O. formosanus*

Time (h)	mortality rate (%)		
	SM1	Mixture of SM1 and <i>B. bassiana</i>	Mixture of SM1 and <i>M. anisopliae</i>
25	46.7±5.8 a	23.3±5.8 b	46.7±5.8 a
26	50.0±0.0 b	26.7±11.6 c	76.8±5.8 a
27	56.7±5.8 b	30.0±0.0 c	76.8±5.8 a
28	63.3±5.8 b	30.0±0.0 c	83.3±5.8 a
29	70.0±0.0 b	46.7±5.8 c	93.3±5.8 a
30	83.3±5.8 b	63.3±5.8 c	100.0±0.0 a
31	83.3±5.8 b	70.0±0.0 c	100.0±0.0 a
32	100.0±0.0 a	76.8±5.8 b	100.0±0.0 a

Means±SD within rows followed by different letters are significantly different (Tukey's test, $P < 0.05$)

pollution-free characteristics suggest that it could be the principal method for controlling *O. formosanus* in the future.

S. marcescens is a naturally occurring ubiquitous bacteria and pathogenic to insects. In recent years, numerous studies have been carried out on *S. marcescens* from various infected insects (e.g. *H. armigera*, *P. rapae*, and *S. exigua*) and significant progress has been made (Liu et al. 1988; Chen et al. 2001; Wang et al. 2010). However, the study of *S. marcescens* for *O. formosanus* control has not been reported previously. The strain SM1 was extracted from *O. formosanus* infected by *S. marcescens*, and toxicity assays with SM1 were carried out. The results show that the SM1 has an obvious toxic effect.

Of the current biological pesticides for *O. formosanus* control, *B. bassiana*, *M. anisopliae* and *B. thuringiensis* were selected to treat *O. formosanus* with 2×10^{10} cells/ml *B. bassiana*, 1×10^{10} cells/mL *M. anisopliae* and 1.6×10^8 IU/mL *B. thuringiensis*. *B. bassiana* and *M. anisopliae* were more toxic. *B. bassiana* achieved 80% mortality after 72 h, and *M. anisopliae* 80% after 96 h. These biological control agents can be used in the prevention and control of *O. formosanus* at the early stage of infestation.

The effects of combined biological agents on *O. formosanus* have also not been reported before. In this study, *B. bassiana* (2×10^{10} cells/mL) and *M. anisopliae* (1×10^{10} cells/mL), which had good toxic effects to *O. formosanus*, were combined with SM1 (1.5×10^{10} cells/mL). The toxicity of the combination of *B. bassiana* and SM1 was lower than that of SM1 alone, but the toxicity of the combination of *M. anisopliae* and SM1 was higher than that of SM1 alone. These results show that different biological agents, when combined into different formulations, will produce different results. Therefore, laboratory experiments must be undertaken to choose suitable combinations of biological agents to avoid antagonism between agents.

This study showed that the *S. marcescens* strain SM1 was significantly toxic to *O. formosanus*, but the specific components and mechanisms of this effect need to be further examined. Research has shown that the pathogenesis of *S. marcescens* is mainly due to chitinase enzymes (Regev et al. 1996; Zhang et al. 2000; Xu and Peng 2004; Yin et al. 2004; Jin et al. 2005), and Tao (2006) has shown that the insecticidal protein of *S. marcescens* is a metallic protein that exists in the supernatant of the bacteria. At the same time, both the living bacteria of *S. marcescens* and its secretions are toxic to insects (Yang et al. 2012, 2015). Therefore, a titration experiment on *O. formosanus* using the different constituents of *S. marcescens* fermentation will be carried out in a follow-up study.

Based on this study of the *S. marcescens* strain SM1, this bacterium has considerable potential for the biological control of *O. formosanus* and needs further study. In addition, this experiment includes only an indoor bioassay of the *S. marcescens* strain SM1, while the control effects of SM1 on an entire colony have not been studied and should be the subject of future research.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Babashpour S, Aminzadeh S, Farrokhi N, Karkhane A, Haghbeen K (2012) Characterization of a chitinase (Chit62) from *Serratia marcescens* B₄A and its efficacy as a bioshield against plant fungal pathogens. *Biochem Genet* 50:722–735
- Chen XW, Fan H, Chen R, Yu XJ, Lan HX, Sun BZ (2001) Study of the pathogenicity on pests and the utility in fields of *Serratia marcescens*. *J Tianjin Agric Coll* 8:28–30 (in Chinese)
- Chakraborty U, Chakraborty BN, Chakraborty AP (2010) Influence of *Serratia marcescens* TRS-1 on growth promotion and induction of resistance in *Camellia sinensis* against *Fomes lamaoensis*. *J Plant Interact* 5:261–272
- Fu RJ, Qi XR, Feng K, Xia XR, Tang F (2019) Identification and characteristics of a strain of *Serratia marcescens* isolated from the termites, *Odontotermes formosanus*. *J Nanjing For Univ Nat Sci Ed* 43(1):76–82 (in Chinese)
- Jin H, Ge SR, Tao Y, Ran HY, Liu SG (2005) Identification of a pathogenic strain of locusts and its toxicity and pathology. *Acta Microbiol Sin* 45:172–176 (in Chinese)
- Lavania M, Chauhan PS, Chauhan SV, Nautiyal CS (2006) Induction of plant defense enzymes and phenolics by treatment with plant

- growth-promoting rhizobacteria *Serratia marcescens* NBRI₁₂₁₃. *Curr Microbiol* 52:363–368
- Liang YY, Cheng SD, Cheng ZX, Qin HG, Hu SX, Li XM (2012) Advances in research on botanical insecticides for termite control. *Chin J Appl Entomol* 49:778–784 (**in Chinese**)
- Liu ZC, Lu YX, Liu JH, Ren PL (1988) Study on *Serratia marcescens*-pathogenic bacteria of rice planthopper. *Nat Enem Insects* 10:34–38 (**in Chinese**)
- Nobutaka S, Masami N, Ken W, Katsymi A (2005) Synergistic antifungal activity of the culture filtrates of *Serratia marcescens* strain B2 and chemical fungicides against the sclerotia viability of the rice sheath blight pathogen, *Rhizoctonia solani*. *Biocontrol Sci* 10:97–100
- Regev A, Keller M, Strizhov N, Sneh B, Prudovsky E, Chet I, Ginzberg I, Koncz-Kalman Z, Koncz C, Schell J, Zilberstein A (1996) Synergistic activity of a *Bacillus thuringiensis* δ -endotoxin and a bacterial endochitinase against *Spodoptera littoralis* larvae. *Appl Environ Microbiol* 62:3581–3586
- Sampson MN, Gooday GW (1998) Involvement of chitinase of *Bacillus thuringiensis* during pathogenesis in insects. *Microbiology* 144:2189–2194
- Tao K (2006) Studies on purification, gene cloning and expression of insecticidal protein. Doctorial dissertation, Sichuan University, Chengdu, China
- Ting ASY, Meon S, Kadir J, Radu S, Singh G (2010) Induction of host defence enzymes by the endophytic bacterium *Serratia marcescens*, in banana plantlets. *Int J Pest Manag* 56:183–188
- Wang K, Yan PS, Cao LX, Ding QL, Shao C, Zhao TF (2013) Potential of chitinolytic *Serratia marcescens* strain JPP1 for biological control of *Aspergillus parasiticus* and Aflatoxin. *Biomed Res Int*. <https://doi.org/10.1155/2013/397142>
- Wang L, Zhang Z, Ou XK, Zhang JF, Jing YB, Zhang ZY, Cao JX, Li YP, Ma SY, Li RB (2010) Preliminary researches on the aphides bio-control with bacteria. *Guangxi Agric Sci* 41:226–230 (**in Chinese**)
- Xu HG, Peng HY (2004) Delayed effects of chitinase of *Serratia marcescens* S₃ on *Helicoverpa armigera* in different instars. *Acta Microbiol Sin* 44:88–92 (**in Chinese**)
- Yang JY, Cao X, Ji CY, Zhao XF, Zhang MX (2015) Inhibitory effects of *Serratia marcescens* isolate PS-1 on the increase of experimental population of *Spodoptera exigua*. *Chin J Biol Control* 31:501–507 (**in Chinese**)
- Yang Q, Zhou Y, Han GJ, Fang JC (2012) Isolation, identification and active ingredient analysis of two pathogenic bacterial strains isolated from *Spodoptera exigua*. *Jiangsu J Agric Sci* 28:1267–1271 (**in Chinese**)
- Yin HX, Zhang J, Hou RT, Wang JD, Yang ZR (2004) Isolation and identification of a chitinase-producing bacterium and its synergistic effect on locust biocontrol. *Plant Prot* 30:37–41 (**in Chinese**)
- Zhang DF, Yang PZ, Cao LL, Jiang ST (2015) Optimization of shake flask cultivation conditions for prodigiosin production by *Serratia marcescens*. *Food Sci* 36:119–124
- Zhang YJ, Tan J, Lin YQ (2000) The effects on peritrophic membrane of *Helicoverpa armigera* treated by low temperature and chitinase. *Chin J Biol Control* 16:152–155 (**in Chinese**)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.