Prodigiosin isolated from *Serratia marcescens* in the *Periplaneta americana* gut and its apoptosis-inducing activity in HeLa cells

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Abstract. *Serratia marcescens* are considered to be abundant and optimal resources for obtaining prodigiosin, which can be isolated from soil, water, plants and air but rarely from insects. In the present study, a strain of *Serratia marcescens* named WA12-1-18 was isolated from the gut of *Periplaneta americana*, which was capable of producing high levels of pigment reaching 2.77 g/l via solid fermentation and was identified as prodigiosin by ultraviolet, high performance liquid chromatography (LC), Fourier-transform infrared spectroscopy, LC-mass spectroscopy and nuclear magnetic resonance. The apoptotic tumor cells treated with prodigiosin were examined by 4',6-diamidino-2-phenylindole (DAPI) staining assays and transmission electron microscopy. Flow cytometry (FCM) was utilized to measure the apoptotic rate with Annexin V staining and the expression levels of proteins involved in apoptosis, including B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X (Bax) and caspase-3 were determined by western blot analysis and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The experimental results revealed that prodigiosin could inhibit the proliferation of HeLa cells and the half-maximal inhibitory concentration values of prodigiosin in HeLa were 2.1, 1.2 and 0.5 μg/ml over 24, 48 and 72 h, respectively. Furthermore, DAPI staining assays and transmission electron microscopy clearly demonstrated that prodigiosin could induce HeLa cell apoptosis. FCM results revealed that the cell apoptotic rates were 19.7±1.4, 23.7±2.4 and 26.2±2.3% following the treatment with 0.5, 1.0 and 2.0 μg/ml prodigiosin for 48 h, respectively. Western blot analysis and RT-qPCR revealed that prodigiosin could activate apoptosis-associated molecules including Bcl-2, Bax and caspase-3. Therefore, the results of the present study demonstrated that the prodigiosin could induce apoptosis in HeLa cells, which may be associated with the upregulation of Bax and caspase-3, the concomitant downregulation of Bcl-2 levels and also triggering the extrinsic apoptotic signaling pathway.

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Introduction

Serratia marcescens can be isolated from soil, water, plants and air but rarely from insects (1). *Periplaneta americana* (a type of American cockroach) is one of the most common household pests, found around drainage basins, sewage ducts, garbage and wall slots. However in China, it is a traditional Chinese medicine used in anti-inflammatory, antiviral, antioxidant and anticancer treatments (2). It has been reported that the extractive *Periplaneta americana* exerts these effects on cancer cell lines such as human cervical cancer cells (HeLa), human hepatoma cells (Bel-7402) and human lung cancer cells (H125) (3,4). Microorganisms in the gut of insects interact with each other, as well as their hosts and their environment. These interactions are thought to have the capacity to degrade the protein and protect the host against diseases through producing secondary metabolites. *Serratia marcescens*, a Gram-negative bacterium, has been reported to produce a large quantity of prodigiosin (5,6). Prodigiosin (2-methyl-3-pentyl-6- methoxyprodiginine) is a red colored secondary metabolite that belongs to a class of tripyrrole compounds. Prodigiosin has also shown effective antimalarial, antifungal and immunosuppressive activities, as well as anticancer activities (7-9).

In the present study, we successfully isolated a strain of *Serratia marcescens* named WA12-1-18 from the gut of *Periplaneta americana*, which was capable of producing high levels of pigment reaching 2.77 g/l via solid fermentation, which

was identified as prodigiosin by ultraviolet (UV), high performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR), LC-mass spectroscopy (LC-MS) and nuclear magnetic resonance (NMR). The aim of the present study was to analyze the antitumor effect of prodigiosin and investigate the mechanisms underlying apoptosis in HeLa cells *in vitro*.

Materials and methods

Isolation of Serratia marcescens from the gut of Periplaneta americana. All *Periplaneta americana* samples were collected from the dormitories and the outdoor surroundings of Guangdong Pharmaceutical University (Guangdong, China), which were identified morphologically and molecularly. *Periplaneta americanas* were fasted for 3 days and then sacrificed following anesthesia with 100% CO_2 for 2 h. They were then washed with distilled water. Sterilization was conducted with 75% ethanol and 3.5% sodium hypochlorite/water solution followed by 3 washes with 1X sterile phosphate-buffered saline (PBS). The entire gut of the *Periplaneta americana* was extracted from the abdomen and then ground into a slurry. The bacterial fluid was diluted into 0.01, 0.005 and 0.00025 μg/ml by gradient, coated on the agar-solidified Luria-Bertani (LB) medium (Amresco, LLC, Solon, OH, USA), which contained of 75 μg/ml potassium dichromate, and then incubated in the dark at 28˚C for a week (10). The morphology and surface characteristics of the bacterial colonies were examined using a scanning electron microscope. Finally, the red-pigment-producing bacterial strain was named, WA12-1-18.

Molecular identification of Serratia marcescens. The strain WA12-1-18 was cultured in LB medium broth for 2 days with shaking at 37˚C and harvested by centrifugation (12,000 x g at 20˚C for 15 min). DNA was extracted and amplified by polymerase chain reaction (PCR) using the genomic DNA as a template and the bacterial universal primers, 27 forward (5'-GAGTTTGATCACTGGCTCAG-3') and 1492 reverse (5'-TACGGCTACCTTGTTACGACTT-3'). The PCR mixture (25 μl) contained 1 μl of template DNA, 2.5 μl of 10X Taq DNA polymerase buffer, 1.5 μl primers (each) and 18.5 μl ddH_2O. The PCR mixture was then incubated with the following thermocycling conditions: 94˚C for 3 min, followed by 35 cycles of 94˚C for 0.5 min, 58˚C for 0.5 min and 72˚C for 1.5 min, followed by a final extension performed at 72˚C for 10 min (11). The amplification products were purified using a DNA purification kit (Qiagen, Inc. Valencia, CA, USA), and sequencing was performed by Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Phylogenetic trees were then constructed from evolutionary distances using the neighbor-joining method (12).

Extraction of prodigiosin. A single colony was inoculated in 10 ml of LB medium and incubated for 12 h at 37˚C, and then transferred into 200 ml LB medium and incubated for a further 3 days at 37˚C. Then, 250 μl bacterial cultures were spread onto LB solid plates and incubated in the dark at a low temperature (23˚C) for 72 h (13). The lawn plates were harvested and dissolved in ethanol and centrifuged at 10,000 x g for 20 min at 4˚C. The upper layer was collected, dried and re-dissolved in ethylacetate, the pigment was purified by silica gel column chromatography (28x12 cm) with petroleum ether and ethylacetate/n-hexane (40:1, v:v), as described previously (14,15). At the end of these procedures, the pigment fractions were pooled and dried.

Identification of prodigiosin. As described previously (16), prodigiosin was analyzed via HPLC at the absorption wavelength 534 nm using Phenomenex Luna C-182 semipreparative column (250x10 mm; sample, 5 μl; Phenomenex, Torrance, CA, USA) with the Bio-Rad HPLC system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The separation was performed using water (solvent A) and acetonitrile/methanol (1:1; solvent B) mobile phases, and a gradient elution program at 3 ml/min with the following parameters: 0-25 min 15-100% B (linear gradient), 25-35 min 100% B and 35-40 min 15% B to re-equilibrate the column. Fractions containing the targeted compounds were combined and concentrated by solvent evaporation. UV absorption of prodigiosin in methanol at pH 5 and pH 9 was scanned using a spectrophotometer at a range of 200-800 nm. In the FTIR study, FTIR spectra were made on a Nicolet Nexus 670 FT-IR infrared spectrometer. All spectra were taken using the Attenuated Total Reflection method (17). Briefly, prodigiosin was mixed with potassium bromide in a clean glass pestle and compressed to produce a pellet. Scanning was performed following base line correction by setting a wave number range of 400-4,000/cm^{-1}. In LC-MS analysis, the pigment was dissolved in acetonitrile, and 10 μl was analyzed in API-4000 Q trap LC-MS/MS. The operating conditions were as follows: 4,500 ionization voltage, 350˚C ionization temperature, ionization mode ESI positive, a MS C18 (50x4.6 mm) column was used, 90:10 (acetonitrile:water) mobile phase, and the injection volume was 10 μl with a flow rate of 0.5 ml/min; performed as described previously (18). In addition, prodigiosin dissolved in d-chloroform was analyzed by hydrogen (^{1}H)-NMR and carbon-13 (^{13}C)-NMR to identify the structure of the purified product. NMR characterization (^{1}H NMR or ^{13}C NMR) was performed using the pulse program zg30 with a spectral width of 8,278.146 Hz using Bruker Advance 400 MHz NMR spectrophotometer (Bruker Corporation, Ettlingen, Germany) with 5 mm probe in $CDCl_3$ solvent.

Detection of the cell proliferation assay of prodigiosin. The HeLa cell line (Experimental Animal Center of Sun Yat-sen University, Guangzhou, China) was maintained in Dulbecco's modified Eagle's medium. An MTT assay was used to measure the levels of cell proliferation. A total of $5x10^4$ cells were treated with different concentrations (0, 0.5, 1.0 and 2.0 μg/ml) of prodigiosin for 24, 48 and 72 h at 37˚C, followed by incubation in MTT (0.1 mg/ml) at 37˚C for 4 h and dissolution in dimethyl sulfoxide at room temperature for 10 min. Microplate reader (at 570 nm) was used to measure the absorbance of each well. Three independent experiments were performed (19-21).

DAPI staining and transmission electron microscopy (TEM). In order to view the nucleus, $5x10^4$ HeLa cells were fixed in methanol, incubated with DAPI for 10 min at 37˚C (Nanjing

KeyGen Biotech Co., Ltd., Nanjing, China; cat. no. KGA215-10), and analyzed using a fluorescence microscope (22). HeLa cells ($5x10^5$) were treated with different concentrations of prodigiosin (0, 0.5, 1.0 and 2.0 μg/ml) for 24 h in 6-well plate. The HeLa cells were then harvested, washed with PBS and fixed with 2.5% glutaraldehyde at 4˚C for 12 h. Following washing with PBS, HeLa cells were dehydrated with ethanol and acetone, sequentially fixed with 1% osmium tetroxide (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 2 h at 37˚C, followed by embedding in epoxy resin for 12 h at 37˚C. Ultra-thin slices (80 nm) were then produced, stained with 1.0% uranylacetate for 5 min at room temperature and viewed on a transmission electron microscope (23,24).

Detection of apoptosis by flow cytometry. Since the emission wavelength of prodigiosin was similar to that of propidium iodide, the levels of apoptosis were detected using Annexin V. HeLa cells ($5x10^5$) were treated with prodigiosin in a concentration- (0, 0.5, 1.0 and 2.0 μg/ml) and time (24, 36 and 48 h)-dependent manner at 37˚C. Following the different exposure times, cells were washed with PBS and resuspended in 150 ml of binding buffer, which contained 5 ml of Annexin V-fluorescein isothiocyanate (Biolegend Inc., San Diego, CA, USA; cat. no. 640912) and were incubated at room temperature for 15 min. The cells were analyzed using a FACScan flow cytometer and the associated software (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

Detection of the apoptotic pathway using western blotting and reverse transcription-quantitative PCR (RT-qPCR). Western blotting was performed as previously described (25). The human caspase-3 antibody (cat. no. 9662) was purchased from Bioworld Technology, Inc. (St. Louis Park, MN, USA), and the human B-cell lymphoma 2 (Bcl-2; cat. no. 2872), Bcl-2-associated X (Bax; cat. no. 2774) antibodies and β-actin (cat. no. 8457; dilution of all primary antibodies; 1:1,000) were purchased from Bosterbio Biological Technology (Pleasanton, CA, USA). The membranes were washed with Tris-buffered saline with 0.1% Tween-20 (TBST) 3 times and incubated with secondary horseradish peroxidase-conjugated antibodies (Anti-rabbit Immunoglobulin G; cat. no. 7074; Thermo Fisher Scientific, Inc.; dilution, 1:5,000) at 25˚C for 2 h. Membranes were then washed with TBST 3 more times and immune reactive protein bands were detected using an enhanced chemiluminescence kit (Beijing Transgen Biotech Co., Ltd., Beijing, China). Image Quant TL 7.0 software (GE Healthcare, Chicago, IL, USA) was employed to quantify protein expression levels. For RT-qPCR, following the supplier's protocol, TRIzol™ reagent (Promega Corporation, Madison, WI, USA) was used to extract the total RNA of HeLa cells that were treated with different concentrations (0, 0.5, 1.0 and 2.0 μg/ml) of prodigiosin, as aforementioned. RNA was quantitated by optical density measurement using a spectrophotometer. The RT-for-PCR Kit (Promega Corporation) was used for RT-PCR. Briefly, 1 μg total RNA with 1 μl 20 μM oligo(dT)18 primers was heated at 70˚C for 2 min, then quenched rapidly on ice. The RT reaction was followed by the addition of 4 μl 5X Reaction buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM $MgCl_2$), 1 μl dNTP mixture (10 mM each dATP, dGTP, dCTP and dTTP), 20 U recombinant RNase inhibitor and 200 U MMLV reverse transcriptase in a 20 μl reaction volume. Samples were incubated at 42˚C for 1 h in an air incubator, followed by inactivation of the reverse transcriptase at 95˚C for 5 min. Then, 80 μl nuclease-free water was added to a final volume of 100 μl. RT-qPCR was performed using primer pairs for Bcl-2, Bax and GAPDH (Table I) with the SYBR-Green PCR Master mix (Promega Corporation) with the following thermocycling conditions: 1 cycle at 94˚C for 4 min, followed by 20 cycles at 94˚C for 30 sec and 68˚C for 1.5 min, and finally 1 cycle at 68˚C for 5 min. The ABI PRISM 7000 sequence detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.) and the $2^{-\Delta\Delta Cq}$ (26) method were used for quantitative analysis.

Table I. List of oligonucleotides used in the present study.

Primer name	Primer sequence (5'-3')
Bcl-2F	CTGTGGATGACTGAGTACC
Bcl-2R	CAGCCAGGAGAAATCAAAC
Bax-F	CTGACATGTTTTCTGACGGCAA
Bax-R	GAAGTCCAATGTCCAGCCCA
GAPDH-F	CTCTGCTCCTGTTCGAC
GAPDH-R	ACGACCAAATCCGTTGACTC

Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-asssociated; F, forward; R, reverse.

Statistical analysis. SPSS 19.0 statistical software (IBM Corp., Armonk, MA, USA) was used to evaluate the results. One-way analysis of variance was used for comparisons between multiple groups. For post hoc analyses, heterogeneity of variance was accepted if $P>0.05$, and the Least Significant Difference method was used. Otherwise, Dunnett's T3 method was used. Homogeneity of variance of the samples to be compared was tested using a Levene's test. The results were expressed as the mean ± standard deviation. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of Serratia marcescens in the gut of Periplaneta americana. Observation of morphological characteristics revealed that the colony of strain surface was smooth, convex and shiny with a vivid red color (Fig. 1A). A straight pole shape with blunt ends and uneven surface waves were observed via micromorphological analysis (Fig. 1B). The 16S r DNA sequences of the red colony (comprising 1,562 nucleotides) were obtained and submitted to EzBioCloud (www.ezbiocloud.net/identify). The sequence exhibited the highest similarity (99.59%) to that of *Serratia marcescens*. A phylogenetic tree was constructed based on the 16S r DNA PCR products as well as on the coding gene sequences of the isolate and its nearest relatives (Fig. 1C). The strain was identified as *Serratia marcescens* and named WA12-1-18.

Extraction and identification of prodigiosin. The red coating of the solid medium was collected following culture for 72 h

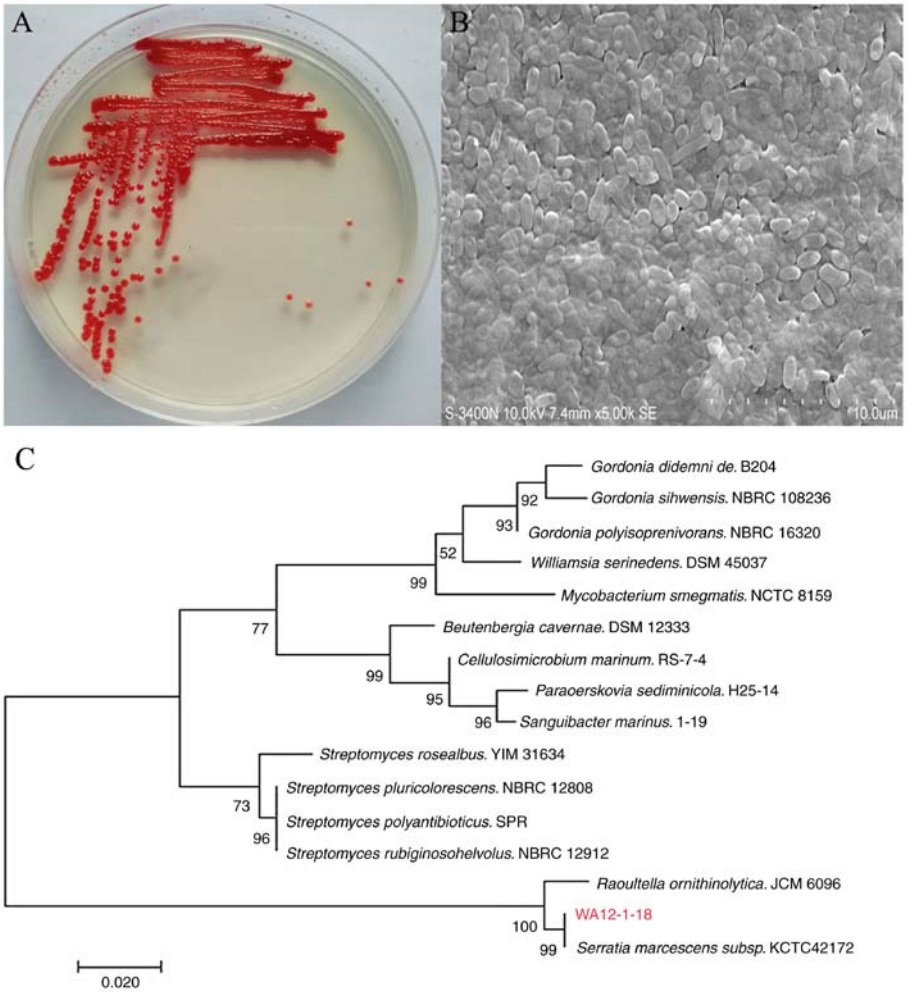

Figure 1. Identification of *Serratia marcescens* from *Periplaneta americana* gut. (A) Morphological characteristics in Luria-Bertani medium broth. (B) Morphological characteristics as revealed by scanning electron microscopy (magnification, x5,000). (C) Phylogenetic analysis of cloned 16S r DNA sequences.

at 23˚C. The solubility test demonstrated that the dark-red powder had good solubility in polar solvent (MeOH and EtOAc) but rarely dissolved in water (Fig. 2A and B). The yield of prodigiosin was high, reaching 2.77 g/l, and the purity of this secondary metabolite produced by *Serratia marcescens* was 98.25% determined by HPLC (Fig. 2C). UV spectrophotometry revealed that the maximum absorptions were at 534 nm (pH=5.0) and 466 nm (pH=9.0; Fig. 2D). The FTIR peak assignments showed that the characteristic wave numbers (cm^{-1}) were at 3,450 (N-H str aromatic), 2,924 and 2,853 (C-H_2 str aliphatic), 1,729 (C-O str ether), and 1,631 and 1,581 (C=C, C=N str aromatic; Fig. 2E). The molecular mass determined by LC-MS was 324.207(m/z 324.207; Fig. 2F). The chemical shifts of prodigiosin were further confirmed by NMR spectroscopy (Fig. 2G and H): ^{1}H-NMR (CDCl3, 300 MHz, ppm) δ 0.98 (3H, t, H11''), 1.29 (2H, m,H9''), 1.45 (2H, m, H10''), 1.59 (2H, m, H8''), 2.38 (2H, t, H7''), 2.47 (3H, s, H6''), 3.31 (1H,m, H3), 4.29 (1H, d, H3'), 4.88 (3H, s, OCH3), 5.10 (1H, brd, H3''), 5.34 (1H, m, H4), 6.37 (1H, brs, H6'),7.18 (1H, m, H2), 7.62 (1H, brs, H1), 7.71 (1H, brs, H1'); ^{13}C-NMR (CDCl3, 75 MHz, ppm) δ 12.03 (C6''), 14.59 (C11''), 23.78(C10''), 23.90 (C7''), 26.79 (C8''), 30.94 (C9''), 37.84 (C3'), 59.57 (OCH3), 66.83 (C3), 95.46 (C6'), 108.79 (C4), 112.46 (C5'), 117.04 (C5), 123.67 (C2''), 128.35 (C2), 130.03 (C3''), 131.02(C4''), 132.50(C5''), 154.30 (C2'), and 169.22 (C4'). All characteristics corresponded to those of prodigiosin ($C_{20}H_{25}N_3O$).

Detection of the cell proliferation assay of prodigiosin. Cell growth of prodigiosin-treated HeLa cells was measured by the MTT assay at different time points over 72 h of the treatment period. The results shown in Table II demonstrated a time- and dose-dependent decline in number of viable cells within 72 h of prodigiosin-treatment. Using the dose-response curves, the half-maximal inhibitory concentration (IC_{50}) values of prodigiosin in HeLa were 2.1 μg/ml at 24 h, 1.2 μg/ml at 48 h and 0.5 μg/ml at 72 h, respectively.

DAPI staining and TEM. The results of the DAPI staining assay revealed that the number of apoptotic cells increased with the prodigiosin concentration following 24 h (Fig. 3A). The morphology of control HeLa cells at 0 μg/ml prodigiosin was used as the normal morphology (Fig. 3B). TEM revealed rich cell surface microvilli, normal nucleus and abundant cytoplasm; the endoplasmic reticulum can also be seen clearly. However, apoptosis was induced when HeLa cells were treated with 2 μg/ml prodigiosin (Fig. 3B), presenting no cell surface

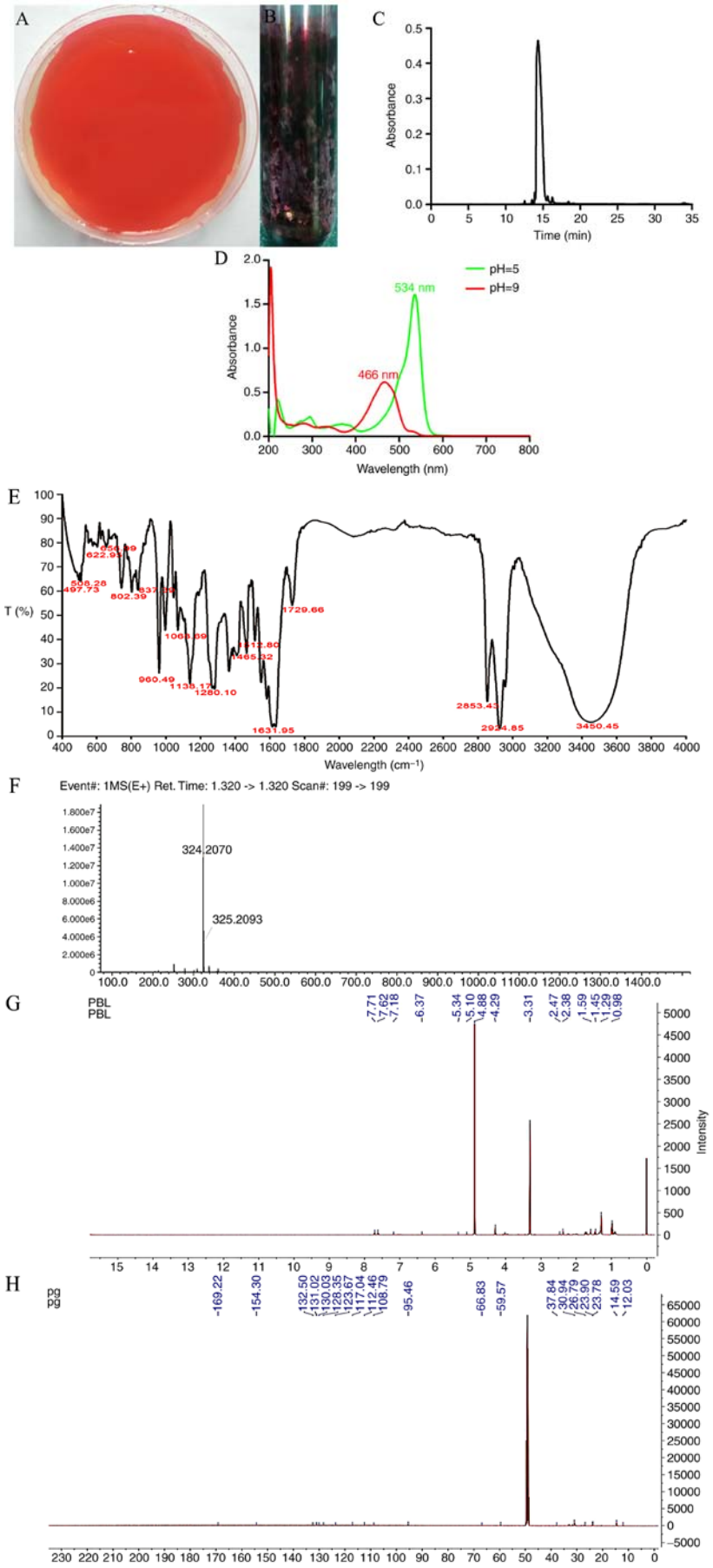

Figure 2. Extraction and identification of prodigiosin. (A) *Serratia marcescens* spreading onto the Luria-Bertani solid plate and incubated in the dark at a low temperature (23˚C) for 72 h. (B) Dry prodigiosin. (C) Purity test of prodigiosin by high performance liquid chromatography. (D) Ultraviolet absorption test of prodigiosin. (E) Fourier-transform infrared spectroscopy study of prodigiosin. (F) Liquid chromatography-mass spectroscopy analysis of prodigiosin. (G) Hydrogen-NMR and (H) carbon-13-NMR analysis of prodigiosin. NRM, nuclear magnetic resonance.

Table II. Inhibition effect of prodigiosin on HeLa cells by MTT assay.

	24 h		48 h		72 h	
Groups	Inhibition rate (%)	P-value	Inhibition rate (%)	P-value	Inhibition rate (%)	P-value
Control	0	-	0	-	0	-
0.5 μg/ml	14.3±2.7[a]	0.040	25.5±1.2[a]	0.007	41.5±1.2[b]	0.005
1.0 μg/ml	26.3±3.1[a]	0.039	40.7±2.3[b]	0.006	74.7±2.3[b]	0.003
2.0 μg/m	50.3±1.6[b]	0.006	63.5±1.0[c]	<0.0001	86.5±1.0[c]	0.002
4.0 μg/ml	57.8±2.9[b]	0.026	84.4±0.1[c]	<0.0001	90.4±1.1[c]	<0.0001
8.0 μg/ml	88.1±2.3[c]	<0.0001	92.3±2.2[c]	<0.0001	95.3±2.2[c]	<0.0001
IC_{50}	2.1 μg/ml		1.2 μg/ml		0.5 μg/ml	

According to the Shapiro-Tilk test, these results have a normal distribution, so one-way analysis of variance was used for comparisons between multiple groups. [a]P<0.05, [b]P<0.01 and [c]P<0.001 vs. control group. IC_{50}, half-maximal inhibitory concentration.

Table III. Effect of prodigiosin on the apoptosis of HeLa cell as determined by flow cytometry.

Groups	24 h Apoptosis rate (%)	P-value	36 h Apoptosis rate (%)	P-value	48 h Apoptosis rate (%)	P-value
Control	1.2±0.5	-	2.8±1.1	-	2.6±0.6	-
0.5 μg/ml	3.8±0.7	0.068	8.5±1.2[a]	0.043	19.7±1.4[b]	0.006
1.0 μg/ml	6.2±1.2[a]	0.046	10.9±1.9[b]	0.008	23.7±2.4[b]	0.003
2.0 μg/m	7.6±1.1[a]	0.039	14.2±1.8[b]	0.007	26.2±2.3[b]	0.001

According to the Shapiro-Tilk test, these results have a normal distribution, so one-way analysis of variance was used for comparisons between multiple groups. [a]P<0.05 and [b]P<0.01 vs. control group.

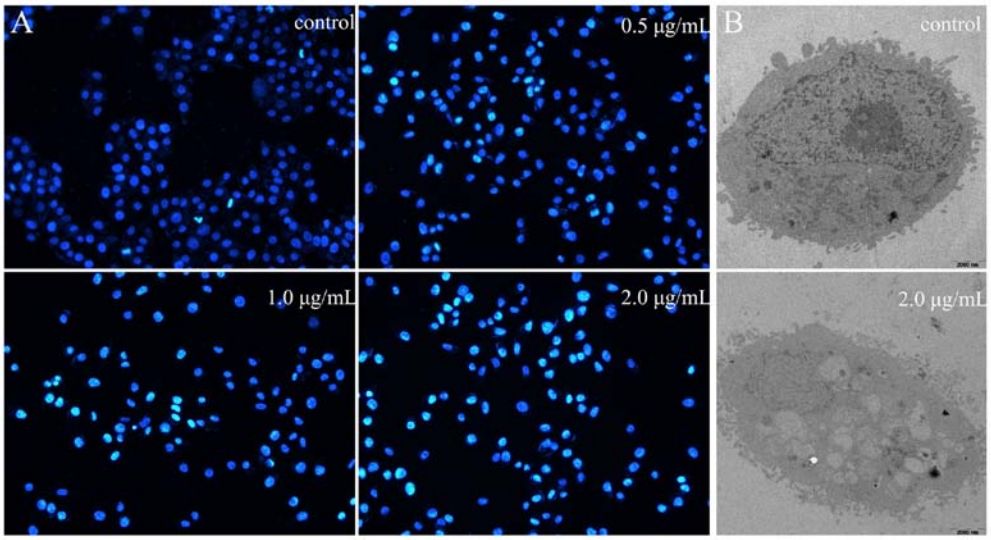

Figure 3. Effect of prodigiosin on HeLa cells as determined by DAPI staining and electron microscopy over 24 h. (A) DAPI staining (magnification, x100) and (B) scanning electron microscopy (magnification, x15,000). DAPI, 4',6-diamidino-2-phenylindole.

microvilli, folded nuclear membrane, increased nuclear heterochromatin and condensed chromatin. These phenomena suggested that the apoptotic process was induced by prodigiosin treatment.

Detection of apoptosis using flow cytometry. To gain further understanding of the mechanism underlying the inhibition of proliferation, the present study investigated the effect on apoptosis by flow cytometry. HeLa cells were treated with

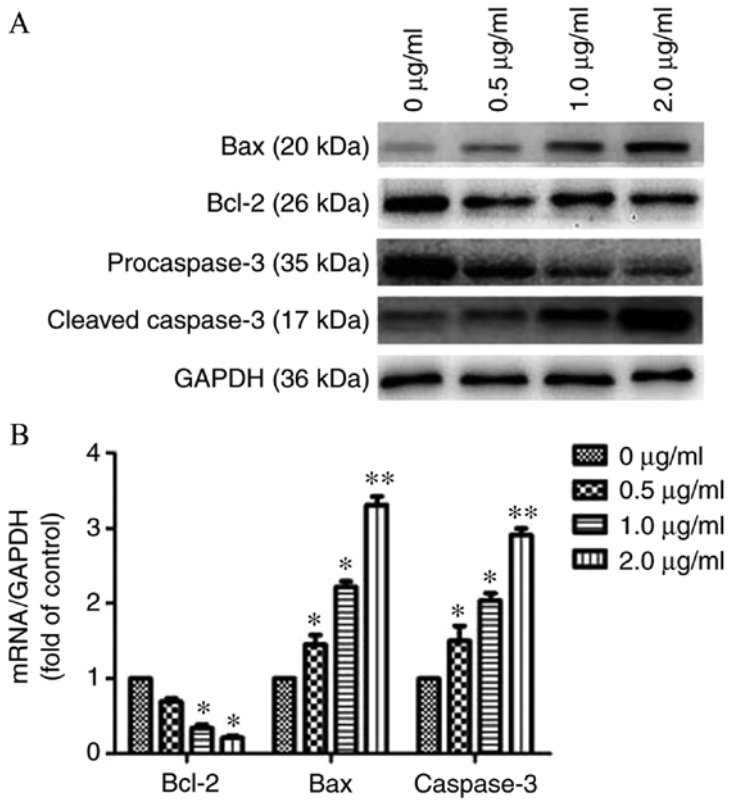

Figure 4. Effect of prodigiosin on anti-apoptotic and proapoptotic gene expression. (A) The protein expression of Bcl-2, Bax and Caspase-3 on HeLa cells as determined by western blotting. (B) Reverse transcription-quantitative polymerase chain reaction detection of Bcl-2 and Bax mRNA levels using total RNA prepared from HeLa cells. *P<0.05 and **P<0.01 vs. control group (0 μg/ml). Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-asssociated X.

0, 0.5, 1.0 and 2.0 μg/ml of prodigiosin for 24, 36 and 48 h, respectively. Annexin V stained cells were tested by flow cytometry for quantitation. There were Annexin V-negative and Annexin V-positive on the dot-plots, as well as the stages of apoptosis located on the upper quadrant of the dot-plot (data not shown). The results shown in Table III demonstrated that there were dose- and time-associated increases in the apoptosis of HeLa cells following prodigiosin treatment.

Detection of the apoptotic pathway using western blotting and RT-qPCR. To further elucidate the mechanism of apoptosis induced by prodigiosin, the present study evaluated the protein and mRNA levels of key regulators via western blotting and RT-qPCR. The results demonstrated that prodigiosin treatment could downregulate Bcl-2 levels, and concomitantly upregulate Bax and caspase-3 levels when compared with the control (0 μg/ml; Fig. 4A and B).

Discussion

The intestinal microbiota of insects has become a key area of interest in recent years (27). *Periplaneta americanas* are insects of the order *Blattodea*, some of which are associated with the human environment and carrier numerous microorganisms (28). There are few studies focusing on the endophytic bacteria in *Periplaneta americana*, especially *Serratia marcescens*. The microbial community in the gut is responsible for digestion, but how they participate and carry out this activity is unknown. The results of the present study therefore confirm the idea that the *Periplaneta americana* gut may be a specialized microhabitat of enhanced microbial activities (29). In the present study, the 16S r DNA gene based phylogenetic tree analysis showed the highest similarity (99.59%), confirming that *Serratia marcescens* was successfully isolated from the gut of *Periplaneta americana*. The authors hope that more novel microorganisms can be isolated in the group's future research. The Gram-negative bacterium *Serratia marcescens* is known for its ability to produce numerous secondary metabolites (30). Prodigiosin is one of the bioactivity secondary metabolites produced by *Serratia marcescens*. Low productivity of prodigiosin was reported under liquid fermentation (7). Therefore, in the present study, prodigiosin was obtained by solid fermentation. Compared with submerged fermentation, solid fermentation is simpler and more efficient in terms of the preparation of prodigiosin (31). Furthermore, the yield of prodigiosin from the gut of the cockroaches was 2.77 g/l, which was higher than that obtained from the body of a grasshopper and the common *Serratia marcescens* (32,33). It is possible that the intestinal bacteria may have the capacity to protect the host against diseases by producing secondary metabolites. The purity of prodigiosin was as high as 98.25%, as determined by HPLC, which was consistent with a previous study (34).

The structure of prodigiosin is similar to that of obatoclax, which is a Bcl-2 inhibitor (35). In the present study, prodigiosin treatment in a HeLa cell line was investigated and the results revealed that prodigiosin inhibited the proliferation of HeLa cells in a time- and dose-dependent manner,

and the IC_{50} values were 0.5-2.1 μg/ml over 24, 48 and 72 h. In addition, another study showed that the A549 cell line was very sensitive to the prodigiosin analogue, with an IC_{50} value of ~2.2 mg/l (36). The results of the DAPI staining assay, flow cytometry and TEM demonstrated that prodigiosin levels increased in the apoptotic cell population in a dose- and time-dependent manner. The possible anticancer mechanisms of prodigiosin reviewed by Sruthy *et al* (29) is attributed as pH modulators, cell cycle inhibitors, DNA cleavage agents and mitogen activated protein kinase regulators. The present results suggested that prodigiosin-induced apoptosis may be due to the down regulation of Bcl-2 levels and the concomitant upregulation of Bax and caspase-3 levels in HeLa cells through the mitochondria pathway; these results were different from those of a previous study (37). Some studies have shown that prodigiosin from the supernatant of *Serratia marcescens* could induce apoptosis as an immunosuppressor in hematopoietic cancer cell lines (38). In addition, there is also evidence that prodigiosin-induced apoptosis could be associated with Bcl-2 and survivin inhibition in HT-29 cells (14). The most notable finding was that prodigiosin had nearly identical cytotoxic effects on the resistant cells when compared with their parental lines (14). An *in vitro* study also revealed that p53 was not induced by prodigiosin in Jurkat and HL-60 cells (39). These results suggest that prodigiosin induces apoptosis independently of p53 and DNA damage.

In conclusion, to the best of the our knowledge, the present study is the first to demonstrate that prodigiosin produced by *Serratia marcescens* in the *Periplaneta americana* gut has functions associated with inhibiting HeLa cell proliferation and promoting apoptosis. Therefore, prodigiosin isolated from *Serratia marcescens* from *Periplaneta americana* may represent a novel class of anticancer agent. However, due to the specific mechanism of apoptosis and complex regulation, the role of prodigiosin in apoptosis mechanisms requires further research to provide a theoretical and practical foundation for the future use of prodigiosin in cancer treatment.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

XBJ designed the experiments. PBL, JS, PYO, LYL and ZYC conducted the experiments. FJC and JW analyzed the experimental results. XBJ and PBL wrote the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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