Polish Journal of Microbiology
2019, Vol. 68, No 2, 185–191
https://doi.org/10.33073/pjm-2019-018

ORIGINAL PAPER

Evaluation of The Pathogenic Potential of Insecticidal *Serratia marcescens* Strains to Humans

EDYTA KONECKA, JOANNA MOKRACKA, SYLWIA KRZYMIŃSKA and ADAM KAZNOWSKI

Department of Microbiology, Faculty of Biology, Adam Mickiewicz University in Poznań, Poland

Submitted 27 November 2018, revised 17 January 2019, accepted 19 January 2019

Abstract

We observed the death of insect caterpillars of *Spodoptera exigua* in the laboratory culture line and identified *Serratia marcescens* as the bacterial causative agent of the insect death. We confirmed that *S. marcescens* had insecticidal activity against *S. exigua* and caused high mortality of larvae. The LC_{50} values of *S. marcescens* CFU per 1 cm^2 of insect diet surface were similar for all isolates. Our research reports novel strains with high pesticidal activity as candidates for future research on a new bioinsecticide. As bioinsecticides cannot be harmful to non-target organisms, we determined the pathogenic properties of *S. marcescens* to humans. We proved the ability of *S. marcescens* to damage mammalian epithelial cells. All strains had cytopathic effects to Vero cells with a cytotoxic index ranging from 51.2% ± 3.8% to 79.2% ± 4.1%. We found that all of the strains excreted catecholate siderophore – enterobactin. All isolates were resistant to sulfamethoxazole, tobramycin, gentamicin, cefepime, and aztreonam. We did not observe the ESBL phenotype and the integrons' integrase genes. Resistance to sulfamethoxazole was due to the presence of the *sul1* or *sul2* gene. The use of resistant *S. marcescens* strains that are pathogenic to humans in plant protection may cause infections difficult to cure and lead to the spread of resistance genes. The results of our study emphasize the necessity of determination of the safety to vertebrates of the bacteria that are proposed to serve as biocontrol agents. The novelty of our study lies in the demonstration of the indispensability of the bacteria verification towards the lack of hazardous properties to humans.

K e y w o r d s: bioinsecticide, insecticidal activity, pathogenicity, pesticide safety, *Serratia marcescens*

Introduction

Due to the growing resistance of insects to synthetic pesticides, there is an urgent need for new biopreparations. *Serratia marcescens* is a species of Gram-negative rods (Hejazi and Falkiner 1997) that produces substances useful in different branches of industry and medicine (Siva et al. 2012). Some *S. marcescens* strains have been recognized as opportunistic or facultative pathogens of insects. They cause lethal septicemia after penetration into the hemocoel. The first step of pathogenesis is the colonization of epithelial cells. After adhesion to the cells, the bacteria produce potential virulence factors, including extracellular toxins which are probably the most common factors of *S. marcescens* pathogenicity (Grimont and Grimont 2006). The pathogenicity of *S. marcescens* to insects makes these bacteria an interesting tool in the search of the new biological control preparation applied in plant protection. Liquid culture of *S. marcescens* had potential use as a biocontrol factor against insect pest of Lepidoptera *Plutella xylostella* (Jeong et al. 2010), *Heliothis virescens* (Sikorowski et al. 2001) and *Helicoverpa armigera* (Mohan et al. 2011). Additionally, *S. marcescens* could reduce the development of fungus and thus inhibit the appearance of some diseases of crops. The bacteria have an antagonistic effect towards plant pathogen belonging to oomycetes – *Phytopthora parasitica* – the causative agent of a disease known as gummosis (de Queiroz and de Melo 2006). It has been demonstrated that the whole *S. marcescens* cells (Sikorowski et al. 2001; Jeong et al. 2010; Mohan et al. 2011) as well as their isolated components (Khanafari et al. 2006; Patil et al. 2011; Aggarwal et al. 2015) had putative usefulness in biocontrol on condition that they are safe for non-target organisms.

As the whole bacterial cells could have a harmful effect on vertebrates, more desirable are the components produced by bacteria. The bioactive substances that may play an important role in plant protection produced by *S. marcescens* include several virulence agents:

* Corresponding author: E. Konecka, Department of Microbiology, Faculty of Biology, Adam Mickiewicz University in Poznań, Poland; **e-mail: edkon@amu.edu.pl**

prodigiosin (Patil et al. 2011) and chitinase (Mohan et al. 2011; Aggarwal et al. 2015) among them. Prodigiosin synthesized by *S. marcescens* is active against insects of Coleoptera (Bidari et al. 2018) and Diptera. It has also medical utility due to antitumor, immunomodulating, antimycotic (Khanafari et al. 2006), and antibacterial features (Samrot et al. 2011). It induces apoptosis in some cancer cell lines, such as acute human T-cell leukemia hepatocellular cancer, breast cancer, and the TNF-stimulated cervix carcinoma (Khanafari et al. 2006). It can also be used as an inhibition agent of protozoan species (Grimont and Grimont 2006). Moreover, *S. marcescens* synthesizes substances active against *Pectobacteria* spp. that causes the soft rot disease of plant *Amorphophallus konjac* (Wu et al. 2012). None of the previous studies on the usefulness of *S. marcescens* in plant protection included the determination of pathogenic potential to humans of *S. marcescens* strains and evaluation of their safety to vertebrates.

The same factors which make *S. marcescens* the putative biological control organisms are these that in humans could facilitate bacterial cells adhesion, protect from host's immune system and allow for tissue penetration. The pathogenicity factors include the fimbriae, the production of siderophores, the presence of cell wall antigens, the ability to resist to the bactericidal action of serum, the production of proteases and the cell-contact toxins that facilitate bacterial invasion, induce cytotoxicity and apoptosis of host cells (Franczek et al. 1986; Krzymińska et al. 2012).

Additionally, the antibiotic resistance of the strains should be considered due to the possibility of the spread of the genes conferring drug resistance. Antibiotic resistance genes are often incorporated into mobile structures such as plasmids, transposons, and integrons. The latter are genetic structures that capture and incorporate resistance gene cassettes and transform them into the functional genes (Cambray et al. 2010).

We have observed the death of beet armyworm *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) larvae in the laboratory culture line of this insect pest. This encouraged us to isolate and identify the bacterial causative agent of the insects' death and evaluate its utility in plant protection against pests. We estimated the 50% lethal concentration (LC_{50}) of the microorganisms for *S. exigua* larvae to confirm the pathogenicity of the bacterial strains for insects. According to the Integrated Pest Management program (Directive 2009/128/WE of the European Parliament and the Council of the European Union), application of insecticidal biopreparations should come with the lowest risk to human health (Matyjaszczyk 2018). Biological pest management can be aiming to maintain food and human safety (Mosa et al. 2016). Therefore, we decided to evaluate if the bacteria that caused the insect death are safe for humans. We examined the cytotoxic activity of the isolates to mammalian epithelial cells and the ability to produce siderophores. Moreover, the strains were verified for antimicrobial susceptibility and the presence of integrons.

Experimental

Materials and Methods

Bacterial strains. Eight *S. marcescens* isolates (MPU Si1 – MPU Si8) were used in this study. They were isolated from dead caterpillars of *Spodoptera exigua* cultured in the laboratory culture line in September 2009. In order to determine the clonal relationship of isolates, thirteen additional strains were included in the study as a comparative samples: *S. marcescens* strain MPU Si9 obtained from a dead larva of *Agrotis segetum* reared in the same laboratory three months earlier, eight *S. marcescens* strains (MPU S11, MPU S14, MPU S15, MPU S26, MPU S32, MPU S40, MPU S41, MPU S47) isolated from clinical specimens, one *S. odorifera* (MPU Se128/7) cultured from sewage, one *S. ficaria* (MPU S49), one strain of *Enterobacter cloacae* (MPU E39a) from clinical specimens, and *Pseudomonas aeruginosa* (MPU Pb5/4) from the intestinal tract of a wild boar.

Bacteria isolation and identification. Dead caterpillars were swabbed with 90% ethanol, homogenized in BHI medium (Difco) and incubated at 30°C for 24h. Then, the bacterial suspension was spread on BHI agar medium and after 24-hour incubation, the bacteria were characterized based on the morphology of colonies. Identification of bacteria was performed by API 20E tests according to the manufacturer's instruction (bioMérieux, France).

Clonal relationship. Bacterial typing was conducted by REP-PCR with primers REP1I and REP2I for repetitive extragenic palindromic sequences (Moura et al. 2007). One bacterial colony was suspended in 25 µl of sterile distilled water and heated at 98°C for 5 min. Two microliters of DNA were added to PCR mixture containing PCR buffer with $NH_4(SO_4)_2$, 0.5 µM of each primer (Oligo.pl, Poland), 200 µM of dNTP mix, 2.5 mM of $MgCl_2$, and 0.5 U of Allegro DNA polymerase (Novazym, Poland). PCR amplification involved an initial denaturation at 94°C for 3 min followed by 35 cycles at 94°C for 1 min, 40°C for 1 min, 72°C for 90, and a final extension at 72°C for 8 min.

Amplification products were electrophoresed in 1.5% NOVA Mini agarose gel (Novazym, Poland). The similarity of bacteria DNA profiles was estimated with Dice coefficient and a dendrogram was created by the unweighted pair group method with average linkages (UPGMA).

Pathogenicity of *S. marcescens* strains for insects. The pathogenicity of *S. marcescens* strains against *S. exigua* caterpillars was determined. Insects originated from laboratory culture line reared at 26°C, 40–60% relative humidity, and a 16:8 (light: dark) period in the Department of Microbiology, Adam Mickiewicz University, Poznań.

The bioassay on insects was conducted according to Jeong et al. (2010), Mohan et al. (2011), and Sikorowski et al. (2001). *S. marcescens* isolates were grown in nutrient agar (Biocorp, Poland) at 30°C for 24 h. One bacterial colony was suspended in 1 ml of 0.85% NaCl. Five different volumes: 2.5 µl, 5 µl, 10 µl, 20 µl, 40 µl of the suspension were spread on pieces (5 mm diameter, 3 mm hight) of semisynthetic diet for *S. exigua* rearing as described by McGuire et al. (1997). The pieces of diet were placed separately in transparent polystyrene multi-well plates. Caterpillars in L1 instar were placed onto the diet – one larva per one well with one diet piece. Each *S. marcescens* CFU number was tested against 30 larvae (three replications with 10 insects each). Simultaneously, the number of CFU per milliliter was determined by the spread plate method. The volume of 100 µl of a bacterial colony suspension in 1 ml of 0.85% NaCl, and additionally the volume of 100 µl of five dilutions of the suspension (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) were spread on BHI agar (each dilution on two plates with medium). After the overnight incubation at 37°C, the colonies were counted and the number of CFU per milliliter was calculated. As we mentioned above different volumes of the suspension were spread on pieces (5 mm diameter, 3 mm height) of semisynthetic diet for insect rearing. The pieces had a shape of a cylinder and the formula for the surface area is πr^2. Knowing the value of the surface area in cm^2, the volume of bacterial suspension spread on the medium, and the number of CFU per milliliter, we calculated CFU/cm^2.

As a positive control, a strain of *Bacillus thuringiensis* subsp. *kurstaki* HD1 from biopesticide Foray was used. Foray contains bacterial endospores (spores) and protein crystals and is dedicated for plant protection against lepidopteran pests. The *B. thuringiensis* was cultured in medium developed for bacteria sporulation by Lecadet and Dedonder (1971). Spores and crystals were applied to insects in the same way as *S. marcescens*. As a negative control, 0.85% NaCl was used.

Infected insects were reared at 26°C, 40–60% relative humidity, and a 16:8 (light: dark) period. The number of dead insects was estimated after 7 days. The LC_{50} [*S. marcescens* CFU per 1 cm^2 of insect diet surface] against insects was calculated by a probit analysis according to Finney, based on the mortality (%) in the control sample, by using BioStat ver. 5.8.4.3 software (AnalystSoft Inc.).

Cytotoxic activity to epithelial cells by MTT assay. African monkey kidney (Vero) cells were cultured in Eagle Minimum Essential Medium (EMEM, Sigma) with 5% fetal calf serum (FCS, Sigma) containing 2 mM glutamine, 50 IU of penicillin per milliliter, streptomycin (100 µg/ml) and nystatin (1 mg/ml). The cells were seeded (1×10^4 per well) and incubated at 37°C in an atmosphere with 5% CO_2.

The strains were cultured in Luria-Bertani medium (LB, Difco) at 37°C for 24 h with shaking at 300 rpm. The supernatants were centrifuged at $3000 \times g$ for 30 min and sterilized through 0.22 µm-pore size membrane filters Millex-GV (Millipore) (Krzymińska et al. 2010).

The epithelial cell monolayer was incubated with bacterial culture filtrates for 24 h at 37°C. As a negative control, the cells were infected with a nonpathogenic *E. coli* K-12C600 supernatant. The cytotoxicity was assessed quantitatively by monitoring the mitochondrial reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) (Krzymińska et al. 2009). The data were presented as means ± standard deviation (SD) from two independent experiments performed in duplicate.

Siderophore production. *Serratia* sp. strains were verified for the production of siderophores by chrome azurol S (CAS) assay (Schwyn and Neilands 1987). The type of the siderophore excreted was identified in cross-feeding assays with the following indicators: enterobactin indicator – *Salmonella typhimurium* TA 2700, aerobactin and rhodotorulic acid indicator – *Escherichia coli* LG 1522, rhizoferrin and α-keto acids indicator – *Morganella morganii* SBK 3 (Reissbrodt and Rabsch 1988), and yersiniabactin indicator – *Yersinia enterocolitica* 5030 (Haag et al. 1993). Additionally, the presence of siderophore receptors for yersiniabactin, aerobactin and glycosylated enterobactin – salmochelin, encoded respectively by the *fyuA*, *iutA* and *iroN* genes, was assessed by PCR. All PCR reagents were purchased from Novazym (Poland). PCR amplification conditions and sequences of primers have been previously published (Karch et al. 1999; Johnson et al. 2000; Johnson and Stell 2000). The PCR products were separated in 1.5% agarose gel. All experiments were performed in triplicate.

Antimicrobial susceptibility. The susceptibility of the isolates to 20 antibiotics representing nine classes was determined according to the standard disk diffusion method recommended by The European Committee on Antimicrobial Susceptibility Testing (EUCAST 2014). The antimicrobials comprised: amikacin (30 µg), tobramycin (10 µg), netilmicin (10 µg), gentamicin (10 µg), ticarcillin (75 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), cefotaxime (5 µg), ceftaroline (5 µg), ceftazidime (10 µg), cefoperazone (10 µg), cefepime (30 µg), co-trimoxazole (25 µg), trimethoprim (5 µg), sulfamethoxazole (200 µg), piperacillin (30 µg), piperacillin/

tazobactam (30–60 μg), aztreonam (30 μg), imipenem (10 μg), and meropenem (10 μg). The production of extended spectrum β-lactamases (ESBL) was determined in the double-disc synergy test. All antibiotic discs were provided by Oxoid. Amplifications of three genes conferring resistance to sulfonamides (*sul1*, *sul2*, *sul3*), were conducted in a 25-μl volume with PCR buffer with $NH_4(SO_4)_2$, 0.5 μM of each primer (Oligo. pl), 200 μM of dNTP mix, 2.5 mM of $MgCl_2$, 0.5 U of DreamTaq polymerase (Thermo Scientific), and 200 ng of genomic DNA. The PCR conditions consisted of initial denaturation at 94°C, 3 min, followed by 35 cycles at 94°C for 45 s, annealing (varied; 46–60°C) for 45 s, 72°C for 90 s, with a final extension at 72°C for 7 min (Pei et al. 2006).

Presence of integrons. The integron integrase genes were detected by multiplex PCR with primers targeting three classes of the integrase genes *intI1*, *intI2* and *intI3* (Dillon et al. 2005). PCR amplifications were performed in a 25-μl volume with 2.5 μl of 10 × PCR buffer with $NH_4(SO_4)_2$, 0.25 μM of each primer, 100 μM of dNTP mix, 2.5 mM of $MgCl_2$, 1 U of DreamTaq polymerase (Thermo Scientific), and 200 ng of genomic DNA. Amplification involved an initial denaturation (94°C, 5 min) followed by 30 cycles of denaturation (94°C, 1 min), annealing (59°C, 1 min) and extension (72°C, 1 min), with a final extension step (72°C, 8 min).

Results

Bacteria identification. The strains isolated from insects were Gram-negative rods identified as *Serratia marcescens* with API 20E system. On the agar medium, the bacteria formed circular, raised, shiny, and smooth colonies. All isolates produced the pigment that made the colonies red.

Clonal relationship. Molecular typing revealed three groups of bacteria with 100% similarity of DNA patterns (Supplementary Fig. S1). Cluster 1 consisted of three isolates: MPU Si4, MPU Si5, and MPU Si7. Three strains MPU Si1, MPU Si2, and MPU Si3 formed cluster 2. Isolates MPU Si6 and MPU Si8 were gathered in cluster 3. These groups comprised *S. marcescens* isolates cultured from dead *S. exigua* insects. The similarity between cluster 1 and 2 was equal to 92%. Group 3 was similar to 1 and 2 in 85%. *S. marcescens* MPU Si9 isolated from the intestinal tract of *A. segetum* cultured in the same laboratory as *S. exigua* but in different time showed 66% similarity with *S. marcescens* isolates originated from *S. exigua*. DNA pattern of *S. marcescens* isolated from *A. segetum* was similar in 70% to the strain cultured from a clinical specimen.

Pathogenicity of *S. marcescens* strains for insects. *S. marcescens* isolates revealed insecticidal activity against *S. exigua* caterpillars and caused high mortality of larvae. The LC_{50} values of *S. marcescens* were similar and ranged from 7.6×10^5 to 5.6×10^6 CFU per 1 cm^2 of the insect diet surface. The toxicities of *S. marcescens* strains were higher than the activity of spore-crystal preparation of *B. thuringiensis* from Foray bioinsecticide (Table I).

Table I
Pathogenicity of *S. marcescens* and *B. thuringiensis* to *S. exigua*.

Strain	LC_{50} (CFU/cm^2)	95% Fiducial limits
MPU Si1	4×10^6	$2 \times 10^6 - 4.3 \times 10^7$
MPU Si2	4.2×10^6	$2 \times 10^6 - 1.1 \times 10^8$
MPU Si3	5×10^6	$2.1 \times 10^6 - 3.8 \times 10^8$
MPU Si4	2×10^6	$5.6 \times 10^5 - 7.6 \times 10^6$
MPU Si5	4.5×10^6	$9.2 \times 10^5 - 2.3 \times 10^7$
MPU Si6	5.6×10^6	$9.2 \times 10^5 - 3.3 \times 10^7$
MPU Si7	7.6×10^5	$2 \times 10^5 - 2.8 \times 10^6$
MPU Si8	2.5×10^6	$1.3 \times 10^6 - 2.4 \times 10^7$
B. thuringiensis	1.7×10^7	$3.6 \times 10^6 - 3 \times 10^7$

Cytotoxicity of *S. marcescens* strains. After 24 h of incubation with the strain culture supernatants, we observed cytopathic effects such as rounding and shrinking of the Vero cells. These changes were followed by the gradual destruction of the monolayer. All strains were cytotoxic to Vero cells (Table II). The highest cytotoxicity in a range from 76.6 to 79.2% was observed for five strains. Three isolates revealed cytotoxic activity, causing death from 54.9 to 59.7% of Vero cells. The supernatant of non-pathogenic control, *E. coli* K-12C600 did not cause the cytopathic effect.

Siderophore production. In the standard method of Schwyn and Neilands (1987), all *S. marcescens* strains demonstrated the production of iron chelators. Biological assays with indicator strains deficient in siderophore synthesis but capable of acquiring exogenous iron chelators indicated that all of the strains excreted catecholate siderophore – enterobactin. The PCR results showed that the strains did not have the receptor genes for yersiniabacin, aerobactin or salmochelin.

Table II
Cytotoxic activity of *S. marcescens* strains isolated from insects.

Strain	Cytotoxic index (%)
MPU Si1	79.2 ± 4.1*
MPU Si2	77.6 ± 2.8
MPU Si3	77.5 ± 3.1
MPU Si4	58.5 ± 2.5
MPU Si5	76.6 ± 1.9
MPU Si6	78.3 ± 3.7
MPU Si7	59.7 ± 2.1
MPU Si8	54.9 ± 3.8

* Mean percentage of cytotoxicity ± SD, measured by MTT assay

Table III
Antibiotic resistance of *S. marcescens* strains isolated from insects.

Isolate	Antimicrobial resistance profile
MPU Si1	SUL*
MPU Si2	SUL
MPU Si3	SUL
MPU Si4	SUL
MPU Si5	TOB, FEP, SUL
MPU Si6	SUL
MPU Si7	GEN, TOB, SUL
MPU Si8	SUL

*ATM – aztreonam, GEN – gentamicin, SUL – sulfamethoxazole, TOB – tobramycin

Antimicrobial susceptibility and presence of integrons. The strains were resistant toward one to three antimicrobials (Table III). All isolates were resistant to sulfamethoxazole. Moreover, they were resistant to antibiotics belonging to aminoglycosides: tobramycin (22.2%) and gentamicin (11.1%), cephalosporins: cefepime (11.1%) and monobactams: aztreonam (11.1%). We did not observe an ESBL phenotype and the integron integrases genes. Resistance to sulfamethoxazole of all strains was due to the presence of the *sul1* or *sul2* gene.

Discussion

In recent years some microbial insect pathogens have become useful in protecting crops against pests. Application of natural enemies of insects could be the long-term and suitable strategy. There is still a need to search for an effective and safe biopreparation against harmful crop pests, and entomopathogens of hazardous insects could be employed in the development of the biopreparations (Bahar and Demirbağ 2007; Nuñez-Valdez et al. 2008). *S. marcescens* strains are proposed to be used as biocontrol agents because of their insecticidal activity against lepidopteran larvae (Sikorowski et al. 2001; Jeong et al. 2010; Mohan et al. 2011) of all developmental stages (Aggarwal et al. 2015). However, the proposal mentioned above ignored the issue of bacterial safety for humans and other non-target vertebrates. We observed the death of insect caterpillars of *S. exigua* and *A. segetum* in the laboratory culture lines. We isolated and identified the bacterial causative agent of dead insect and determined the pathogenic properties of insecticidal *S. marcescens* strains to humans.

For determining the possible clonality of strains, we applied the REP-PCR typing method to determine the genetic similarity of Gram-negative rods (Rasschaert et al. 2005; Henriques et al. 2006; Moura et al. 2007; Czajkowski et al. 2010; Mokracka et al. 2011). The analysis revealed a high similarity of all *S. marcescens* isolates cultured from *S. exigua* intestinal track. DNA profiles of strains that caused infections of *S. exigua* varied significantly from that of the strain infecting *A. segetum*. The strains made three clusters, yet we did not treat them as clones as they differed in resistance profiles and cytotoxic activity.

We confirmed entomopathogenicity of *S. marcescens* isolates. The insecticidal activity of strains indicates the usefulness of the bacteria in plant protection. The low values of LC_{50} of *S. marcescens* against caterpillars in comparison with LC_{50} of spore-crystal preparation of *B. thuringiensis* from Foray suggested the possibility to create a novel biopesticide based on *S. marcescens* with high activity against lepidopteran pests. However, the preparation could not have harmful effect for non-target organisms, so we examined the cytotoxic activity of the isolates to mammalian epithelial cells, determined the siderophore production, antimicrobial susceptibility, and the presence of integrons in *S. marcescens* genomes.

We observed that culture filtrates of *S. marcescens* strains isolated from insects were capable of damaging mammalian epithelial cell line. Escobar et al. (2001) have reported that supernatants of *S. marcescens* isolates from plants and insects were cytotoxic to epithelial cell lines. Strains originated from the stomach of *Rhodnius prolixus* larvae revealed hemolytic activity (Azambuja et al. 2004). Toxin production by entomopathogenic strains is still not clearly defined. Hertle (2005) has suggested that hemolysin produced by *S. marcescens* strains (ShlA) is the major cytolysin in the pathogenesis of the bacteria. The toxin induces the formation of pores in erythrocytes and eukaryotic nucleated cells, which results in osmotic lysis. Moreover, the bacteria produce extracellular lecithinase, proteinase, and chitinase that play a role in their virulence for insects (Grimont and Grimont 2006). Similar cytotoxic destruction of epithelial cells has been observed for strains isolated from human specimens (Krzymińska et al. 2010). The results presented in this study suggested that extracellular factors produced by the isolates could be responsible for the destruction of the epithelial barrier, which could pose a potential risk to human health.

Iron sequestration involving siderophores is a recognized factor essential for bacterial pathogenicity. Deletion of siderophore biosynthetic genes leads to loss of pathogenicity in a mouse infection model, and also affects maturation of biofilms, surface motility, activation of exotoxins and synthesis of other virulence factors (Vokes et al. 1999; Visca et al. 2007; Mossialos and Amoutzias 2009). Enterobactin, which is a prototypical catecholate siderophore with the highest known affinity for iron, was excreted by all *S. marcescens* strains (Supplementary Table SI). Its role in pathogenesis is limited, as in host it is bound by siderocalin (lipocalin 2, Lcn2)

that inhibits bacterial iron acquisition (Flo et al. 2004). Other siderophores like structurally distinct yersiniabactin or salmochelin, which is glycosylated enterobactin, deliver iron to bacteria despite the presence of Lcn2, yet we did not find receptors for these chelators.

We did not find integrons in the genomes in *S. marcescens* strains although all of them were resistant to sulfamethoxazole, suggesting the presence of class 1 integrons, which have the *sul*1 gene at the 3' integron end. The resistance to sulfamethoxazole was determined by the *sul1* or *sul2* genes that code for dihydropteroate synthases, which are not inhibited by sulfonamides (Pei et al. 2006). The resistance of single strains to aztreonam and cefepime may be the result of overproduction of β-lactamase and resistance to aminoglycosides, which is often noted in clinical strains of *S. marcescens*. It is most frequently determined by the presence of the plasmid-mediated aminoglycoside-modifying enzymes. Both the *sul* genes and those conferring resistance to β-lactams and aminoglycosides may reside on mobile genomic elements and be spread *via* horizontal gene transfer (Stokes and Gillings 2011).

Our research confirmed the potential pathogenicity to humans of *S. marcescens* strains lethal for insect pests. We decided to undertake this issue because *S. marcescens* is proposed to be a useful bioinsecticide (Sikorowski et al. 2001; Jeong et al. 2010; Mohan et al. 2011; Aggarwal et al. 2015); however, the pesticidal biopreparations cannot be harmful to non-target organisms. The ability of *S. marcescens* to damage mammalian epithelial cell line and to employ siderophore-mediated strategies of iron acquisition eliminates the bacterial cells to be used as a bioinsecticide. Using the antimicrobial resistant, potentially pathogenic to humans *S. marcescens* strains in plant protection may cause human infections difficult to cure and lead to the spread of resistance genes. We emphasize the necessity of determining the safety to vertebrates of the bacteria that are proposed to be used as a biocontrol preparation for reducing the number of insects. Yet, it is worth mentioning that bacterial strains harmful for pests could be a source of novel genes or factors useful in controlling insect pests. In our further studies, the insecticidal factors-coding genes in *S. marcescens* strains will be identified, cloned and expressed in *E. coli* cells (Baranek et al. 2015). After isolation and purification of the genes' products, their insecticidal activity against lepidopteran pests will be determined.

ORCID
Edyta Konecka 0000-0002-2334-8857

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Aggarwal C, Paul S, Tripathi V, Paul B, Khan MA. Chitinolytic activity in *Serratia marcescens* (strain SEN) and potency against different larval instars of *Spodoptera litura* with effect of sublethal doses on insect development. BioControl. 2015 Oct;60(5):631–640. doi:10.1007/s10526-015-9674-3

Azambuja P, Feder D, Garcia ES. Isolation of *Serratia marcescens* in the midgut of *Rhodnius prolixus*: impact on the establishment of the parasite *Trypanosoma cruzi* in the vector. Exp Parasitol. 2004 May;107(1-2):89–96. doi:10.1016/j.exppara.2004.04.007 Medline

Bahar A, Demirbağ Z. Isolation of pathogenic bacteria from *Oberea linearis* (Coleptera: Cerambycidae). Biologia. 2007 Jan 1;62(1): 13–18. doi:10.2478/s11756-007-0009-4

Baranek J, Kaznowski A, Konecka E, Naimov S. Activity of vegetative insecticidal proteins Vip3Aa58 and Vip3Aa59 of *Bacillus thuringiensis* against lepidopteran pests. J Invertebr Pathol. 2015 Sep;130:72–81. doi:10.1016/j.jip.2015.06.006 Medline

Bidari F, Shams-Bakhsh M, Mehrabadi M. Isolation and characterization of a *Serratia marcescens* with insecticidal activity from *Polyphylla olivieri* (Col.: Scarabaeidae). J Appl Entomol. 2018 Feb; 142(1-2):162–172. doi:10.1111/jen.12421

Cambray G, Guerout AM, Mazel D. Integrons. Annu Rev Genet. 2010 Dec;44(1):141–166. doi:10.1146/annurev-genet-102209-163504 Medline

Patil CD, Patil SV, Salunke BK, Salunkhe RB. Prodigiosin produced by *Serratia marcescens* NMCC46 as a mosquito larvicidal agent against *Aedes aegypti* and *Anopheles stephensi.* Parasitol Res. 2011 Oct;109(4):1179–1187. doi:10.1007/s00436-011-2365-9 Medline

Czajkowski R, de Boer WJ, Velvis H, van der Wolf JM. Systemic colonization of potato plants by a soilborne, green fluorescent protein-tagged strain of *Dickeya* sp. biovar 3. Phytopathology. 2010 Feb;100(2):134–142. doi:10.1094/PHYTO-100-2-0134 Medline

Dillon B, Thomas L, Mohmand G, Zelynski A, Iredell J. Multiplex PCR for screening of integrons in bacterial lysates. J Microbiol Methods. 2005 Aug;62(2):221–232. doi:10.1016/j.mimet.2005.02.007 Medline

Escobar MM, Carbonell GV, Beriam LOS, Siqueira WJ, Yano T. Cytotoxin production in phytopathogenic and entomopathogenic *Serratia marcescens*. Rev Latinoam Microbiol. 2001 Oct-Dec;43(4): 165–170 Medline.

EUCAST. Breakpoint tables for interpretation of MICs and zone diameters, version 4.0. The European Committee on Antimicrobial Susceptibility Testing. 2014.

Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, Akira S, Aderem A. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature. 2004 Dec 16;432(7019):917–921. doi:10.1038/nature03104 Medline

Franczek SP, Williams RP, Hull SI. A survey of potential virulence factors in clinical and environmental isolates of *Serratia marcescens.* J Med Microbiol. 1986 Sep 01;22(2):151–156. doi:10.1099/00222615-22-2-151 Medline

Grimont F, Grimont PAD. The genus *Serratia*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, editors. The Proaryotes, 3rd ed. Berlin (Germany): Springer Verlag; 2006. p. 219–244.

Haag H, Hantke K, Drechsel H, Stojiljkovic I, Jung G, Zähner H. Purification of yersiniabactin: a siderophore and possible virulence factor of *Yersinia enterocolitica*. J Gen Microbiol. 1993 Sep 01; 139(9):2159–2165. doi:10.1099/00221287-139-9-2159 Medline

Henriques IS, Fonseca F, Alves A, Saavedra MJ, Correia A. Occurrence and diversity of integrons and β-lactamase genes among ampicillin-resistant isolates from estuarine waters. Res Microbiol. 2006 Dec;157(10):938–947. doi:10.1016/j.resmic.2006.09.003 Medline

Hertle R. The family of *Serratia* type pore forming toxins. Curr Protein Pept Sci. 2005 Aug 01;6(4):313–325. doi:10.2174/1389203054546370 Medline

Hejazi A, Falkiner FR. *Serratia marcescens*. J Med Microbiol. 1997 Nov 01;46(11):903–912. doi:10.1099/00222615-46-11-903 Medline

Jeong HU, Mun HY, Oh HK, Kim SB, Yang KY, Kim I, Lee HB. Evaluation of insecticidal activity of a bacterial strain, *Serratia* sp. EML-SE1 against diamondback moth. J Microbiol. 2010 Aug; 48(4):541–545. doi:10.1007/s12275-010-0221-9 Medline

Johnson JR, Russo TA, Tarr PI, Carlino U, Bilge SS, Vary JC Jr, Stell AL. Molecular epidemiological and phylogenetic associations of two novel putative virulence genes, *iha* and $iroN_{E.\ coli}$, among *Escherichia coli* isolates from patients with urosepsis. Infect Immun. 2000 May 01;68(5):3040–3047. doi:10.1128/IAI.68.5.3040-3047.2000 Medline

Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis. 2000 Jan;181(1):261–272. doi:10.1086/315217 Medline

Khanafari A, Assadi MM, Fakhr FA. Review of prodigiosin, pigmentation in *Serratia marcescens*. Online J Biol Sci. 2006 Jan 1;6(1): 1–13. doi:10.3844/ojbsci.2006.1.13

Karch H, Schubert S, Zhang D, Zhang W, Schmidt H, Olschläger T, Hacker J. A genomic island, termed high-pathogenicity island, is present in certain non-O157 Shiga toxin-producing *Escherichia coli* clonal lineages. Infect Immun. 1999 Nov;67(11):5994–6001. Medline

Krzymińska S, Mokracka J, Koczura R, Kaznowski A. Cytotoxic activity of *Enterobacter cloacae* human isolates. FEMS Immunol Med Microbiol. 2009 Aug;56(3):248–252. doi:10.1111/j.1574-695X.2009.00572.x Medline

Krzymińska S, Raczkowska M, Kaznowski A. Cytotoxic activity of *Serratia marcescens* clinical isolates. Pol J Microbiol. 2010;59(3): 201–205. Medline

Krzymińska S, Ochocka K, Kaznowski A. Apoptosis of epithelial cells and macrophages due to nonpigmented *Serratia marcescens* strains. Scientific World Journal. 2012;2012:1–8. doi:10.1100/2012/679639 Medline

Lecadet MM, Dedonder R. Biogenesis of the crystalline inclusion of *Bacillus thuringiensis* during sporulation. Eur J Biochem. 1971 Nov;23(2):282–294. doi:10.1111/j.1432-1033.1971.tb01620.x Medline

Matyjaszczyk E. "Biorationals" in integrated pest management strategies. J Plant Dis Prot. 2018 Dec;125(6):523–527. doi:10.1007/s41348-018-0180-6

McGuire MR, Galan-Wong LJ, Tamez-Guerra P. Bioassay of *Bacillus thuringiensis* against lepidopteran larvae. In: Lacey LA, editor. Manual of techniques in insect pathology. San Diego (USA): Academic Press; 1997. p. 91–99.

Mohan M, Selvakumar G, Sushil SN, Bhatt JC, Gupta HS. Entomopathogenicity of endophytic *Serratia marcescens* strain SRM against larvae of *Helicoverpa armigera* (Noctuidae: Lepidoptera). World J Microbiol Biotechnol. 2011 Nov;27(11):2545–2551. doi:10.1007/s11274-011-0724-4

Mokracka J, Koczura R, Jabłońska L, Kaznowski A. Phylogenetic groups, virulence genes and quinolone resistance of integron-bearing *Escherichia coli* strains isolated from a wastewater treatment plant. Antonie van Leeuwenhoek. 2011 May;99(4):817–824. doi:10.1007/s10482-011-9555-4 Medline

Mosa WFAEG, Sas Paszt L, Frąc M, Trzciński P. Microbial products and biofertilizers in improving growth and productivity of apple – A review. Pol J Microbiol. 2016 Aug 26;65(3):243–251. doi:10.5604/17331331.1215599 Medline

Mossialos D, Amoutzias G. Role of siderophores in cystic fibrosis pathogenesis: foes or friends? Int J Med Microbiol. 2009 Feb;299(2): 87–98. doi:10.1016/j.ijmm.2008.06.008 Medline

Moura A, Henriques I, Ribeiro R, Correia A. Prevalence and characterization of integrons from bacteria isolated from a slaughterhouse wastewater treatment plant. J Antimicrob Chemother. 2007 Oct 03;60(6):1243–1250. doi:10.1093/jac/dkm340 Medline

Nuñez-Valdez ME, Calderón MA, Aranda E, Hernández L, Ramírez-Gama RM, Lina L, Rodríguez-Segura Z, Gutiérrez MC, Villalobos FJ. Identification of a putative Mexican strain of *Serratia entomophila* pathogenic against root-damaging larvae of Scarabaeidae (Coleoptera). Appl Environ Microbiol. 2008 Feb 01; 74(3): 802–810. doi:10.1128/AEM.01074-07 Medline

de Queiroz BPV, de Melo IS. Antagonism of *Serratia marcescens* towards *Phytophthora parasitica* and its effects in promoting the growth of citrus. Braz J Microbiol. 2006 Dec;37(4):448–450. doi:10.1590/S1517-83822006000400008

Pei R, Kim SC, Carlson KH, Pruden A. Effect of River Landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). Water Res. 2006 Jul;40(12):2427–2435. doi:10.1016/j.watres.2006.04.017 Medline

Rasschaert G, Houf K, Imberechts H, Grijspeerdt K, De Zutter L, Heyndrickx M. Comparison of five repetitive-sequence-based PCR typing methods for molecular discrimination of *Salmonella enterica* isolates. J Clin Microbiol. 2005 Aug 01;43(8):3615–3623. doi:10.1128/JCM.43.8.3615-3623.2005 Medline

Reissbrodt R, Rabsch W. Further differentiation of Enterobacteriaceae by means of siderophore-pattern analysis. Zentralbl Bakteriol Mikrobiol Hyg A. 1988 May;268(3):306–317. doi:10.1016/S0176-6724(88)80015-4 Medline

Samrot AV, Chandana K, Senthilkumar P, Kumar GN. Optimization of prodigiosin production by *Serratia marcescens* SU-10 and evaluation of its bioactivity. Int Res J Biotechnol. 2011;2:128–133.

Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Anal Biochem. 1987 Jan;160(1): 47–56. doi:10.1016/0003-2697(87)90612-9 Medline

Sikorowski PP, Lawrence AM, Inglis GD. Effects of *Serratia marcescens* on rearing of the tabacco budworm (Lepidoptera: Noctuide). Am Entomol (Lanham Md). 2001;47(1):51–60. doi:10.1093/ae/47.1.51

Siva R, Subha K, Bhakta D, Ghosh AR, Babu S. Characterization and enhanced production of prodigiosin from the spoiled coconut. Appl Biochem Biotechnol. 2012 Jan;166(1):187–196. doi:10.1007/s12010-011-9415-8 Medline

Stokes HW, Gillings MR. Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. FEMS Microbiol Rev. 2011 Sep;35(5):790–819. doi:10.1111/j.1574-6976.2011.00273.x Medline

Visca P, Imperi F, Lamont IL. Pyoverdine siderophores: from biogenesis to biosignificance. Trends Microbiol. 2007 Jan;15(1):22–30. doi:10.1016/j.tim.2006.11.004 Medline

Vokes SA, Reeves SA, Torres AG, Payne SM. The aerobactin iron transport system genes in *Shigella flexneri* are present within a pathogenicity island. Mol Microbiol. 1999 Jul;33(1):63–73. doi:10.1046/j.1365-2958.1999.01448.x Medline

Wu J, Liu X, Diao Y, Ding Z, Hu Z. Authentication and characterization of a candidate antagonistic bacterium against soft rot of *Amorphophallus konjac*. Crop Prot. 2012 Apr;34:83–87. doi:10.1016/j.cropro.2011.12.008

Supplementary materials are available on the journal's website.