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Antimicrobial Activity of Prodigiosin from *Serratia* sp. PDGS^{120915} Against Intestinal Pathogenic Bacteria

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This study aimed to identify and characterize the antimicrobial activity of prodigiosin produced by *Serratia* sp. PDGS^{120915} isolated from stream water in Busan, Korea; the identification was performed using phonological, biochemical, and molecular techniques, including 16S rRNA sequence analysis. Prodigiosin from the bacterial culture was purified by high-performance liquid chromatography (HPLC), and its antimicrobial activity and minimum inhibitory concentrations (MICs) were evaluated against 10 intestinal pathogenic gram-positive and negative bacteria. The results revealed that the isolated prodigiosin exhibited high antimicrobial activity against *Listeria monocytogenes, Bacillus cereus, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus,* and *Vibrio parahaemolyticus*; further, the isolated prodigiosin showed minimum inhibitory concentrations (MICs) between 3 µg/ml and 30 mg/ml, but they were not active against *Bacillus subtilis, Enterococcus faecalis, Klebsiella pneumonia*, and *Escherichia coli*. In conclusion, prodigiosin isolated from *Serratia* sp. PDGS^{120915} showed high antimicrobial activity against intestinal pathogenic bacteria and has potential applications in the development of new antimicrobial agents.

Keywords: **Prodigiosin, antimicrobial, pigment, *Serratia,* FIC index**

Introduction

Many artificial synthetic colorants, which have widely been used in food, dyestuff, cosmetics and pharmaceuticals, comprise various hazardous effects. To counter the ill effect of synthetic colorants, there is worldwide interest in process development for the production of pigments from natural sources [1]. Natural pigments can be obtained from two major sources, plants [2, 3] and microorganisms [4–9]. The advantages of pigments produced from microorganisms include easy and fast growth in the cheap culture medium, independence from weather conditions and colors of different shades. Hence, production of natural pigments from microorganisms is now one of the emerging fields of research to demonstrate its potential for various industrial applications [1].

Serratia sp. are gram negative bacteria, and classified in the large family of *Enterobacteriaceae*. *Serratia* sp. are opportunistic human, plant and insect pathogens and have been isolated from soil, water, air, foodstuff, plant surface and animals [10]. Another characteristic feature of the *Serratia* is the production of prodigiosin [11]. Prodigiosin, a non-diffusible red pigment, is a secondary metabolite formed by the enzymatic condensation of 2-methyl-3-amylpyrrole and 4-methoxy-2,2'-bipyrrole-5-carboxyaldehyde, leading to a tripyrrole derivative, 2-methyl-3-amyl-6methoxyprodigiosene [12]. The pigment has no defined role in the physiology of producing strains, but has been reported to have antifungal, antibacterial, algicidal, antiprotozoal, antimalarial activities, immunosuppressive and anticancer activities [13–17]. Especially, in case of red pigment produced by marine bacterium *Hahella chejuensis* and *Zooshikella ganghwensis*, it suggested that possibility of potential

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natural substances for having immunosuppressant and algicidal activities [18–21]. In the previous our research, isolation and characterization of *Serratia* sp. PDGS^{120915} was reported [22].

In the present study, we have investigated the antimicrobial activity of purified prodigiosin isolated from *Serratia* sp. PDGS^{120915} against various microorganisms, which produced pathogens related to intestinal diseases, including *Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa,* and *Vibrio parahaemolyticus*. In addition, in order to investigate the quantitative tests for antimicrobial activity of the purified prodigiosin, we have measured the MIC (minimum inhibitory concentrations) and MBC (minimum bactericidal concentration). Also, we have elucidated the synergistic effect on the antimicrobial activity between purified prodigiosin and various antibiotics against tested intestinal microorganisms. It will provide a crucial information on the antimicrobial activity of purified prodigiosin.

Materials and Methods

Bacterial strains and medium

The standard bacterial strains used in this study were purchased from the Korean Collection for Type Cultures (KCTC; Korea) and the Korea Culture Center of Microorganisms (KCCM; Korea). All strains were cultivated in appropriate media (Table 1).

Antimicrobial activity against intestinal pathogens

Previous study, we performed the extraction and purification of prodigiosin produced from *Serratia* sp. PDGS^{120915} and reported [22] (Figs. 1 and 2). To study the antimicrobial activity of prodigiosin produced by *Serratia* sp. PDGS^{120915}, purified prodigiosin was inoculated as much as 15 mg, 1.5 mg, 0.15 mg, and 0.015 mg into each paper disc. After dry, it was placed on the surface of the McFarland No. 0.5 of test bacteria. Following 24 h of incubation at each adequate temperature about 25–37℃, the plates were examined for the MIC is defined as the lowest concentration of antimicrobial activity that inhibits visual growth of microorganisms after 24 h incubation at 37℃ [22]. MICs were determined by a two-fold serial dilution method in each medium as described by the National Committee for Clinical Laboratory Standards (NCCLS) [23]. MBC is defined as the lowest concentration of an antimicrobial activity required for a 99.9% reduction in the viable cell population [23]. For MBC determination, it was poured onto agar plate which was taken from a MIC test well that did not show turbidity.

Synergistic effect between prodigiosin and antibiotics

In order to examine the synergistic effects on the antimicrobial activity between the purified prodigiosin and various antibiotics including amikacin (AN), tobramycin (NN), ceftaxidime (CAZ) and ticarcillin/clavulanic acid (TIM) (BD BBL^{TM}) against various intestinal pathogens, we have measured the fraction inhibitory concentration (FIC) [12, 7]. FIC was calculated as following equation.

$FIC_A = MIC_A$ in combination / MIC_A,
$FIC_B = MIC_B$ in combination / MIC_B,
FIC Index = $FIC_A + FIC_B$

FIC index values were; = 0.5, synergic; > to = 1, addi-

Table 1. List of strains and growth conditions used for antimicrobial activity test.

Gram Positive Bacteria	*B. cereus* KCCM 11204	Nutrient Agar (Difco 0001), 30℃
	B. subtilis KCCM 11779	Nutrient Agar (Difco 0001), 30℃
	L. monocytogenes KCCM 40307	Brain Heart Infusion Agar (Difco 0418), 37℃
	E. faecalis KCCM 12448	Brain Heart Infusion Agar (Difco 0418), 37℃
	S. aureus KCCM 11593	Nutrient Agar (Difco 0001), 37℃
Gram Negative Bacteria	*K. pneumonia* KCCM 11418	Nutrient Agar (Difco 0001), 37℃
	E. coli KCTC 1116	Nutrient Agar (Difco 0001), 37℃
	S. typhimurium KCCM 40253	IFO Medium 802, 30℃
	P. aeruginosa KCCM 11266	Nutrient Agar (Difco 0001), 37℃
	V. parahaemolyticus KCCM 11965	Nutrient Agar (Difco 0001) + 3% NaCl, 37℃

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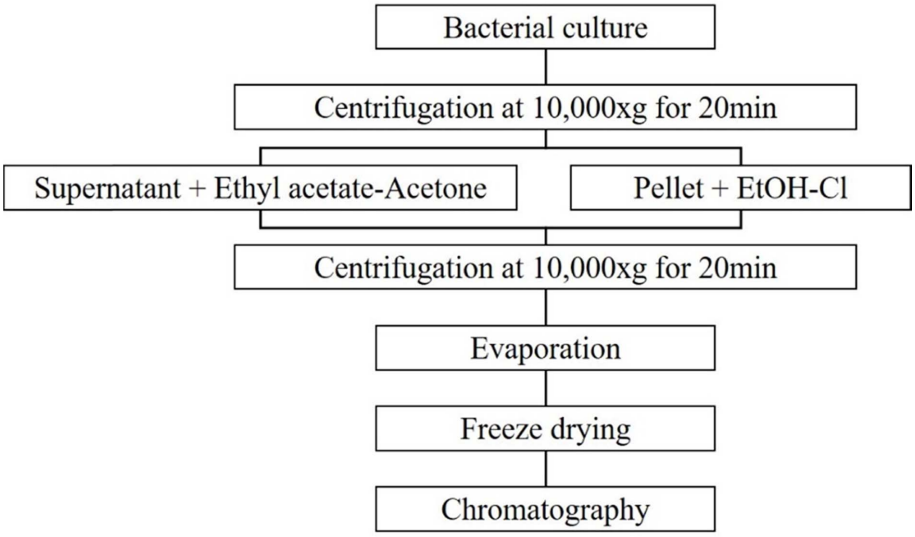

Fig. 1. The procedure for extraction and purification of prodigiosin from *Serratia* sp. PDGS^{120915}.

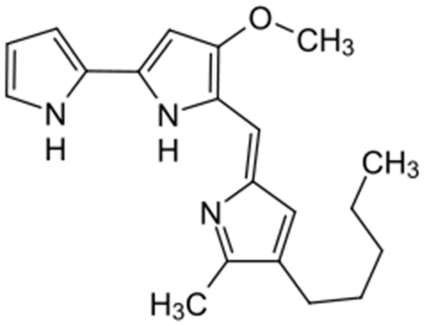

Fig. 2. The chemical structure of prodigiosin.

tive; >1 to = 2, independent and >2, antagonistic.

Results and Discussion

Antimicrobial activity

To investigate the antimicrobial activity of prodigiosin produced by *Serratia* sp. PDGS^{120915}, we have examined the antimicrobial activity of purified prodigiosin against various intestinal pathogenic bacteria. From the results, purified prodigiosin showed a broad spectrum of antimicrobial activity on the tested bacteria as shown in Fig. 3. The previous report, *S. marcescens* showed a higher antimicrobial activity against Gram positive bacteria, including *Staphylococcus, Bacillus, Enterococcus* and *Streptococcus* [24]. However, purified prodigiosin isolated from *Serratia* sp. PDGS^{120915} showed a nonspecific antimicrobial activity on Gram positive and negative pathogenic bacteria. Especially, it showed the highest antimicrobial effects on *L. monocytogenes,* causing infectious listeriosis. The antimicrobial inhibition zone against *L. monocytogenes, B. cereus, P. aeruginosa, S. typhimurium,* and *V. parahaemolyticus* was 44, 37, 30, 28, and 26 mm, respectively as summarized in Table 2.

The results of antimicrobial activities in this study, we observed highly antimicrobial activity against *L. monocytogenes* with treatment 15 mg/ml of purified prodigiosin. In case of listeriosis, it was recommended ceftriaxone 2 g/12 h plus ampicillin 3 g/6 h treatment [25]. Therefore, purified prodigiosin is expect to a new potent antibiotic for the treatment of listeriosis. In addition, purified prodigiosin showed a wide range of antimicrobial activities, and it will provide convenience and efficiency on the treatment of various diseases.

Determination of MIC and MBC

From the results of the MIC and MBC test, purified prodigiosin showed high antimicrobial activity against *L. monocytogenes, B. cereus, P. aeruginosa, V. parahaemolyticus,* and *S. typhimurium*. The MIC values were in the range of 32 to 64 μg/ml (Table 3). The antibacterial activities of purified prodigiosin was evaluated by the MBC assay. As summarized in Table 3, the MBC values were higher than MIC values, especially the lowest MBC value showed in *L. monocytogenes*.

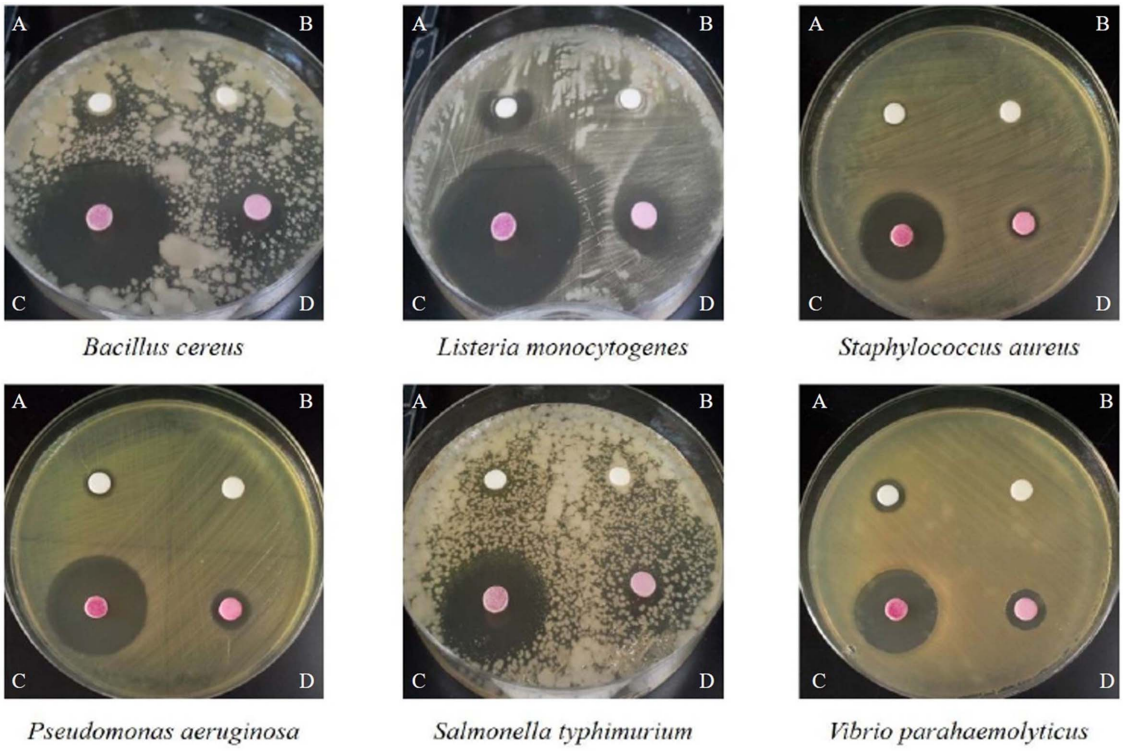

Fig. 3. Antimicrobial effects of prodigiosin from *Serratia* sp. PDGS^{120915} against intestinal pathogens. Each disc contain different concentration of purified prodigiosin. A: 0.15 mg, B: 0.015 mg, C: 15 mg, D: 1.5 mg

The MBC results were indicating that it is possible to inhibition of bacterial growth over at 99% against *B. cereus, L. monocytogenes, S. typhimurium, P. aeruginosa* and *V. parahaemolyticus* when the treatment at 256 μg/ml of purified prodigiosin. Also, it is possible to inhibition of bacterial growth using maximum 64 μg/ml except *L. monocytogenes* at 32 μg/ml.

These results means that the low concentration of purified prodigiosin can do inhibition of growth against corresponding pathogenic bacteria. It is considered that

Table 2. Antimicrobial activity of prodigiosin from *Serratia* sp. PDGS^{120915} against intestinal pathogens.

	Strain	Inhibition zone (mm)			
		15 mg	1.5 mg	0.15 mg	0.015 mg
Gram Positive Bacteria	*B. cereus* KCCM 11204	37	16	0.6	0
	B. subtilis KCCM 11779	0	0	0	0
	L. monocytogenes KCCM 40307	44	17	15	0
	E. faecalis KCCM 12448	0	0	0	0
	S. aureus KCCM 11593	25	0.5	0	0
Gram Negative Bacteria	*K. pneumonia* KCCM 11418	0	0	0	0
	E. coli KCTC 1116	0	0	0	0
	S. typhimurium KCCM 40253	28	12	0.5	0
	P. aeruginosa KCCM 11266	30	13	0.9	0
	V. parahaemolyticus KCCM 11965	26	12	10	0

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Table 3. MIC and MBC of prodigiosin from *Serratia* sp. PDGS^{120915} against intestinal pathogens.

	Strains	MIC (μg/ml)	MBC (μg/ml)
Gram Positive Bacteria	*B. cereus* KCCM 11204	64	256
	B. subtilis KCCM 11779	>1,024	>1,024
	L. monocytogenes KCCM 40307	32	64
	E. faecalis KCCM 12448	>1,024	>1,024
	S. aureus KCCM 11593	512	>1,024
Gram Negative Bacteria	*K. pneumoniae* KCCM 11418	>1,024	>1,024
	E. coli KCTC 1116	>1,024	>1,024
	S. typhimurium KCCM 40253	64	256
	P. aeruginosa KCCM 11266	64	128
	V. parahaemolyticus KCCM 11965	64	128

this will not only reduce the side effects of the overuse of antibiotics, but also enable a decrease in the incidence of antibiotic resistance bacteria.

Determination of FIC indices

The synergistic effects of purified prodigiosin with antibiotics were estimate using the FIC index. These results indicated that the microbial growth suppression was much effective on the combination of the prodigiosin-amikacin and prodigiosin-tobramycin. Also, when the treated with ceftazidime and ticarcillin/clavulanic acid, microbial growth suppression effect was stronger much in the treatment with the combination of prodigiosin-ceftazidime and prodigiosin-ticarcillin/clavulanic acid (Table 4). The results from the present study showed that the combination of purified prodigiosin with certain types of antibiotics showed synergistic or independent effects on pathogenic bacteria. These results provided that the purified prodigiosin could be potential candidate for developing new antibiotics with strong effect against pathogenic bacteria.

Acknowledgments

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

Table 4. MIC and FIC indices of prodigiosin from *Serratia* sp. PDGS^{120915} in combination with antibiotics against intestinal pathogens.

Strains	Amikacin				Tobramycin				Ceftazidime				Ticarcillin/clavulanic acid			
	MIC (μg/ml)			FIC[a]	MIC (μg/ml)			FIC	MIC (μg/ml)			FIC	MIC (μg/ml)			FIC
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
*B. cereus*KCCM 11204	16	8	16	0.75	8	4	16	0.75	16	8	32	1	64	64	32	1.5
L. monocytogenes KCCM 40307	16	2	8	0.375	8	2	2	0.3125	16	4	16	0.75	64	8	16	0.625
S. aureus KCCM 11593	16	4	128	0.5	8	2	128	0.5	16	4	32	0.3125	64	32	256	1
S. typhimurium KCCM 40253	16	4	16	0.5	8	2	4	0.3125	16	8	16	0.75	64	16	8	0.375
P. aeruginosa KCCM 11266	16	4	8	0.375	8	2	4	0.3125	16	4	16	0.5	64	8	8	0.25
V. parahaemolyticus KCCM 11965	16	4	8	0.375	8	2	8	0.375	16	4	16	0.5	64	16	8	0.375

A, without prodigiosin; B, MIC value of antibiotics with prodigiosin; C, MIC value of prodigiosin with antibiotic

[a]The FIC was calculated as the MIC of prodigiosin or each antibiotics in combination divided by MIC of prodigiosin of each antibiotic alone. The FIC index was obtained by the sum of FICs. The FIC index indicated synergy: 0.5, synergic; >0.5 to 1, additive; >1 to 2, independent; >2, antagonistic

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