Study on Antibiotic compounds from *Pseudomonas aeruginosa* NO4 Strain

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1. Introduction

As important human and veterinary medicines, antibiotics are being produced and consumed in large quantities around the world. For example, more than 50 million pounds (22,000 tons) of antibiotics are produced in the U.S. each year and annual production in Germany is about 2,000 tons. Antibiotics are low molecular weight microbial metabolites that at low concentrations inhibit the growth of other microorganisms [1]. Resistant bacteria may also spread and become broader infection-control problems, not only within healthcare institutions, but in communities as well. Clinically important bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) [2, 3]. MRSA is a common cause of infection among hospitalized patients. *Pseudomonas aeruginosa* is a major cause of opportunistic infections among immunocompromised individuals. The spread of this organism in healthcare settings is often difficult to control due to the presence of multiple intrinsic and acquired mechanisms of antimicrobial resistance [4]. In this study, we isolated novel bacterium which had strong antagonistic activity and separated antibiotic compounds from *Pseudomonas* sp., and analyzed characteristics and molecular weight of the antibiotic compound.

2. Methods and Results

2.1 Identification of the antagonistic bacteria

Soil samples were collected from location near Gwangju city. The appropriate amount of the soil samples was suspended in sterile water, and then diluted. The diluted samples were inoculated in the LB(*Luria-Bertani*) agar medium as described in the routine spreading method. The plates were incubated at 37℃ for 12 hours and pure isolates were obtained. Isolation of bacteria named NO4, to identified this bacteria, we analyze 16s rRNA sequence. Genomic DNA was purified using the phenol sampling process and then performed PCR with forward primer(5'-TGG CTC AGA ACG AAC GCT-3') and reverse primer(5'-CCC ACT GCT GCC TCC CGT-3'). Amplified DNA was introduced into *E.coli* DH5α after ligation with pGEMT-easy vector(Promega Co.). And the recombinant plasmid DNA was isolated and sequenced.

2.2 Irradiation test

The samples packed in a conical tube were irradiated with 10 ~ 500 Gy using a ^{60}Co (7.4PBq) irradiator at the Korea Atomic Energy Research Institute.

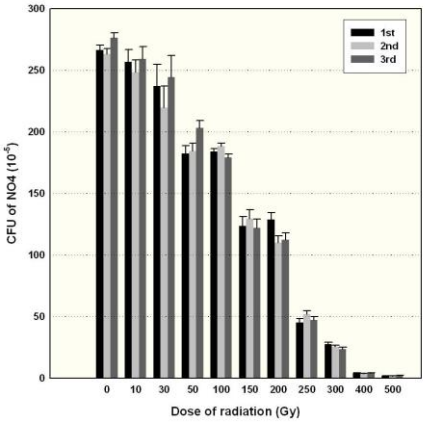


Fig. 1. Survival rate of NO4 at different radiation dose.

2.3 Purification and antibiotic compounds

The antibiotic compounds produced by *Pseudomonas* sp. NO4 were detected by extraction each 4 hours. At 32 hours, the concentration of antibiotic compounds reached its highest level. The antibacterial compounds were isolated and purified according to the scheme shown. The antibiotic activity was determined according to the bioassay method.

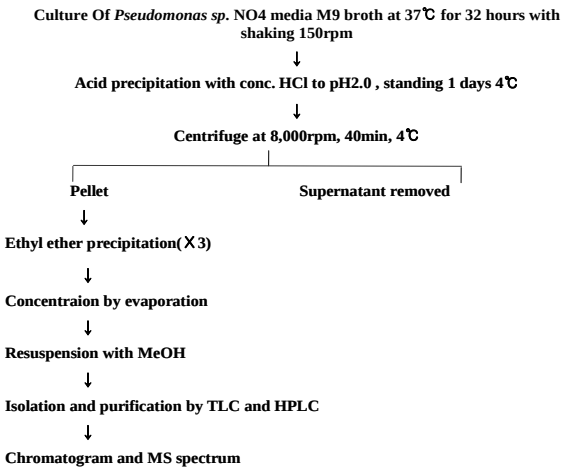


Fig. 2. Process for extraction and purification of antibiotic compounds produced by NO4.

The solution was dried with a rotary evaporator. The obtained pellet was dissolved in methanol, and the solution was fractioned on 60% methanol and filtered with a 0.20 ㎛ non-pyrogenic hydrophilic filter paper (Satorious AG, Goettingen, Germany). Containing

antibiotic compounds were performed by Thin layer chromatography(TLC) and High performance liquid chromatography(HPLC). We could obtain purified antibiotic compounds and active peaks were finally purified by re-HPLC using C18 column with methanol:acetonitrile:30mM Tris = 95:2.5:2.5 in at a flow-rate of 1 ㎖/min and a single compound was finally obtained. Purified compound was detected by GC/MS.

Table I: Antibacterial spectrum of NO4 against various pathogenic bacteria.

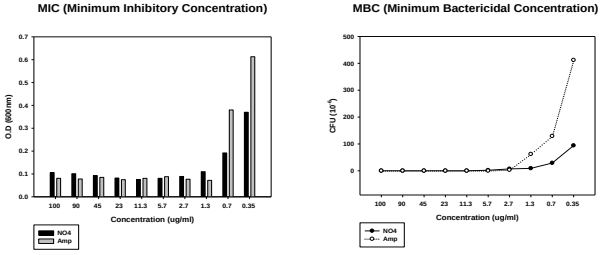
<10mg/mL>

Pathogenic bacteria	G(−) or G(+)	clear zone (mm)	strains inhibited
Edwardsiella tarda	G(−)	9	+++
Escherichia coli O157	"	3	++
Salmonella sp.	"	7	+++
Vibrio ordalii	"	13	++++
Bacillus thuringiensis CMB 26	G(+)	13	++++
Streptococcus iniae 36	"	13	++++
Staphylococcus aureus KACC10778	"	15	++++
Methicillin resistant *Staphylococcus aureus* (MRSA) 1	"	12	++++
MRSA 2	"	13	++++
MRSA 3	"	15	++++
MRSA 4	"	13	++++
MRSA 5	"	8	+++
MRSA 6	"	13	++++
MRSA 7	"	13	++++
MRSA 8	"	14	++++

See Materials and Methods for other experimental details.
(-), not inhibited; ++++: 10~15mm, +++: 6~9 mm, ++: 0~5 mm

2.4 Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

Culture media were prepared, adding antimicrobial crude extracts agents in 2 fold- serial dilution, obtaining final containing 90 to 0.18 ㎍/㎖ of medium. The microdilution tubes were incubated for 12 hours at 37℃. After incubation, the series of dilution tube is observed for microbial growth, usually indicated by turbidity (OD_{600}). MIC indicates the inhibitory potential, while MBC shows the cidal potential of the extracts on MRSA. The MBC is set up with subcultures made from each MIC tube that appears visually clear. The MBC is the concentration of drug lowest concentration that prevented growth of more than one colony on subculture. Results obtained showed that ethyl ether extract of NO4 strain was showing noticeable anti-MRSA MIC and MBC ranging from 0.18 ㎍/㎖, 2.7 ㎍/㎖.



MIC_{50} Amp 0.35 μg/ml
MIC_{50} NO4 0.18 μg/ml

MBC Amp 11.25 μg/ml
MBC NO4 2.7 μg/ml

Fig. 3. MIC and MBC of NO4 crude extracts on MRSA.

3. Conclusions

Antibiotics at high concentrations kill or inhibit the growth of bacterial pathogen, and may act as a single molecule regulating bacterial gene expression and modulating bacterial fitness at subinhibitory concentrations. Antibiotic resistance is a pervasive and growing clinical problem. In this study, we isolated novel bacterium which had strong antagonistic activity and its properties were characterized, against pathogenic bacteria. The LD_{50} of irradiated *P. aeruginosa* NO4 was about 150 to 200 Gy. This antibiotic compound has antibiotic activity to *Bacillus thuringiensis* CMB26, *Streptococcus iniae* 36, *Edward siella tarda*, *Vibrio ordallii*, *Escherichia coli* O157, *Salmonella* sp., *Bacillus subtilis* CMB32 and MRSA. And we isolated antibiotic compounds by TLC, HPLC and characterized of MW of the antibiotic compound was 270 by GC/MS. The crude extracts were screened for the anti-MRSA's potentials using the minimal inhibitory concentration and minimal bactericidal concentration assay. The results suggest that *P. aeruginosa* NO4 could be employed as a novel antibacterial agent in various pathogen and MRSA infection care products. We prepared several antibiotic compounds using supernatant extraction, but all isolated antibiotic compounds were not yet identified. Further research on active compounds and the mode of action of the selected antibiotic is necessary.

ACKNOWLEDGMENT

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