Altering vancomycin susceptibility in vancomycin resistant enterococci by *vanH* promoter and *ddl* gene transformation

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Jun Yong Choi

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Abstract

Altering vancomycin susceptibility in vancomycin resistant enterococci by *vanH* promoter and *ddl* gene transformation

Background: Acquired resistance to vancomycin in enterococci is due to the production of peptidoglycan precursors ending in D-alanyl-D-lactate instead of D-alanyl-D-alanine. In vancomycin resistant enterococci (VRE) the *vanHAXYZ* genes encode a new pathway of enzymes that reduce pyruvate to D-lactate (*vanH*), and combine D-alanine and D-lactate to produce D-alanyl-D-lactate (*vanA*). We investigated the effect of *vanH* promoter and *ddl* gene transformation on vancomycin susceptibility in a VanA phenotype of *Enterococcus faecalis*.

Materials and methods: A 445 bp DNA fragment containing the *vanH* promoter and a 1074 bp fragment containing the putative enterococci *ddl* gene were amplified by PCR. To construct plasmid pJW1, pAM401 DNA was digested with *Sal*I and *Sph*I and ligated to a DNA fragment carrying a *vanH* promoter digested with the same enzymes. Plasmid pJW2 was constructed by cloning the *BamH*I-*Xba*I fragment of the *ddl* gene into pAM401 digested with the same enzymes. To construct pJW3, the *ddl* gene was ligated downstream of the *vanH* promoter after digestion with *BamH*I-*Xba*I and ligation into the same site of the plasmid pJW1 digested with same enzymes. Electrotransformation of the competent VanA phenotype *E. faecalis* with pJW1, pJW2 and pJW3 was accomplished

with a Bio-Rad Gene Pulser. The minimum inhibitory concentration (MIC) of vancomycin for *E. faecalis* and *E. faecalis* transformed with pJW1, pJW2, and pJW3 was determined using the broth dilution method. The expressions of the *vanA* and *ddl* genes of the VanA phenotype of *E. faecalis* and transformed *E. faecalis* were evaluated by RT PCR.

Results: The vancomycin MIC for competent *E. faecalis* was 1024 μg/mL, for *E. faecalis* transformed with pJW1 and pJW3 this was reduced to 256 μg/mL, and the vancomycin MIC for *E. faecalis* transformed with pJW2 was reduced to 512 μg/mL. RT PCR revealed that the *vanA* and *ddl* genes of VRE and *E. faecalis* transformed with pJW1, pJW2, and pJW3 were expressed.

Conclusion: This study presents a way of altering high-level vancomycin resistance with gene transformation in enterococci. The *vanA* and *ddl* gene were expressed in the vancomycin resistant *E. faecalis*. In the future, development of an effective gene delivery system will probably contribute to the design of new modalities that will help overcome the limitations of antimicrobial therapy.

Key Words: enterococcus, vancomycin, vancomycin resistance, transformation, bacteria

Altering vancomycin susceptibility in vancomycin resistant enterococci by *vanH* promoter and *ddl* gene transformation

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I. Introduction

Glycopeptide antibiotics are currently used to treat severe infections due to gram-positive bacteria[1,2]. The rise in drug resistant bacterial infection has made glycopeptide very important in the control of nosocomial infection[3]. It is widely used as the last defense against antibiotics-resistant gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus*, and β-lactam resistant enterococci. Unfortunately, vancomycin-resistant enterococci (VRE) emerged in 1988 in the United Kingdom and France[4,5] and has become one of the major nosocomial pathogens in United States and Europe. In Korea, the proportion of VRE among all enterococcal isolates has rapidly increased since 1992 and VRE has now become one of the major

nosocomial pathogens[6,7].

Vancomycin functions by binding to the cell wall via D-alanyl-D-alanine dipeptide on the peptidoglycan chain terminal, and it inhibits essential cell wall synthesis, thereby inducing cell lysis. Acquired resistance to glycopeptide antibiotics in enterococci is due to the production of peptidoglycan precursors ending in D-alanyl-D-lactate depsipeptide instead of the D-alanyl-D-alanine dipeptide. LeClercq *et al.*[4] demonstrated that high level resistance to vancomycin in enterococcus is plasmid mediated, and Arthur *et al.*[1] characterized transposon 1546 related elements that are responsible for the dissemination of vancomycin resistance gene clusters. The *vanHAXYZ* genes in transposon 1546 encode enzymes that reduce pyruvate to D-lactate (*vanH*), combine D-alanine and D-lactate to produce D-alanyl-D-lactate (*vanA*), and then hydrolyses the normal metabolite D-alanyl-D-alanine while sparing D-alanyl-D-lactate (*vanX*)[8,9]. The reprogramming of peptidoglycan to end in D-alanyl-D-lactate rather than the normal D-alanyl-D-alanine has no effect on the crosslinking efficiency of the transpeptidating penicillin binding proteins, but the switch from the D, D-dipeptide terminus to the D, D-depsipeptide terminus lowers the binding affinity of vancomycin by 1,000-fold[8,10], and enables the VRE to grow at 1,000-fold-higher levels of antibiotics. Transcription from the *vanH*, *-A*, and *-X* genes with in

the *vanA* operon is under the control of the *vanH* promoter and is induced by the binding of the phosphorylated gene product of *vanR* [11,12]. Phosphorylation of the *vanR* response regulator enhances the affinity of the protein for the regulatory regions of the *vanR* and *vanH* promoters and allows transcription of the regulatory (*vanRS*) and resistant (*vanHAXYZ*) genes[13-15].

Many attempts have been made to overcome the resistance of VRE. For example, Walsh *et al.* tested specific inhibitors of *vanA*, *H*, and *X*, which were very effective in vitro, but they did not enter intact bacterial cells. In addition, new antimicrobial agents such as linezolid or quinupristin/dalfopristin have recently been licensed in the United Kingdom and the United States.

Historically, the discovery of new antimicrobials has induced the emergence of pathogens that have new resistance patterns. In addition, we live in an era when antimicrobial resistance has spread at an alarming rate. Recent reports of vancomycin intermediate *S. aureus* in Japan and the United States suggest that common, invasive, microbial pathogens have the potential to become refractory to any chemotherapeutic agent in the future[16,17]. In attempts to inhibit pathogens that have become resistant to conventional antimicrobial agents, gene-based strategies have been studied, although primarily in eukaryotic systems[11,18,19].

Here, we investigate the effect of a *vanH* promoter and of a *ddl* gene transformation on vancomycin susceptibility in a VanA phenotype vancomycin resistant *Enterococcus faecalis*.

II. Materials and Methods

1. Bacterial strains, plasmids

Plasmid pAM401 (American Type Culture Collection, Rockville, Md. USA) was used as the cloning vector for DNA fragments. *Escherichia coli* DH5α was the host strain for recombinant plasmids. Recombinant plasmids were introduced by electrotransformation into *E. faecalis* containing pIP816 (the strain was a gift from P. Couvalin at the Pasteur Institute).

2. Plasmid construction

Plasmid DNA isolation, digestion with restriction endonucleases, amplification of DNA by polymerase chain reaction (PCR) with Taq DNA polymerase, ligation of DNA fragments with T4 DNA ligase, nucleotide sequencing with T7 DNA polymerase, and transformation of *E. coli* DH5α with recombinant plasmid were performed by using standard methods[20].

A colony of VRE was inoculated into 50 μL of distilled water in microcentrifuge tube, which was boiled at 100 ℃ for 5 minutes and centrifuged for 5 minutes. Supernatant obtained from the centrifuged microcentrifuge tube was considered to contain the total DNA of VRE.

The primers used in the *vanH* promoter PCR corresponded to bases 5593 to 5610 and bases 6003 to 6020 in the *vanA* resistance gene cluster

(table 1. GenBank assession number M97297), and the primers used in the enterococci *ddl* gene PCR to bases 33 to 52 and bases 1060 to 1079 in *E. faecalis* putative ddl gene (table 1. GenBank accession number U00457). PCR was carried out with 1 μL of heat-extracted template DNA, 20 pmol of each primer, and PreMix(AccuPover PCR PreMix, Bioneer, Daejeon, Korea) containing 1 μg of Taq DNA polymerase in a total volume of 20 μL. Model 2400 PE Thermal Cycler (Applied Biosystems, San Hose, CA, USA) was used for DNA amplification carried out at 94 ℃ for 5 minutes followed by 30 cycles at 94 ℃ for 1 minute, 56 ℃ for 1 minute, and 72 ℃ for 1 minute. A band at 445 bp was identified for *vanH* promoter PCR, and 1074 bp for the *ddl* gene PCR (figure 1). The amplified DNA was purified by gel extraction using a commercial kit (QIAquick gel extraction kit, Qiagen, USA).

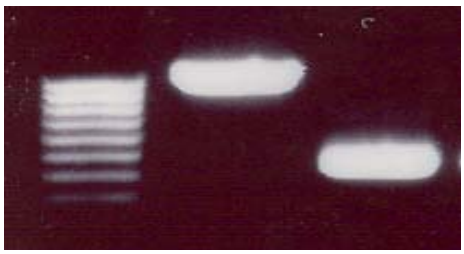

Figure 1. PCR of *vanH* promoter and *ddl* gene. A band at 445 bp was detected for the *vanH* promoter PCR, and 1074 bp for the *ddl* gene PCR. (a. 100 bp ladder, b. *ddl* gene, c. *vanH* promoter)

Table 1. PCR primers for *vanH* promoter and *ddl* gene amplification

5'-3' DNA sequence	Use	Coordinate
catgtcgacagataagctagctgccat	*vanH* promoter sense	5593-5610[1]
gtgcatgccataattaagaccaaccc	*vanH* promoter antisense	6003-6020[1]
gtggatccttgaagattattttgttgta	putative *ddl* sense	33-52[2]
actctagattatttaaaacgattcaaagct	putative *ddl* antisense	1060-1079[2]

[1] Coordinates are based on the published Tn1546 sequence (GenBank accession number M97297).

[2] Corresponds to the published sequence of the putative *ddl* gene of *E. faecalis* (GenBank accession number U00457)

Nucleotide sequences that are underlined represent modifications made to generate the cohesive ends for cloning.

Plasmids pJW1, pJW2, and pJW3 were derivatives of the shuttle vector pAM401. To construct plasmid pJW1, pAM401 DNA was digested with *Sal*I and *Sph*I and ligated with a DNA fragment carrying the *vanH* promoter digested with the same enzymes. Plasmid pJW2 was constructed by cloning the *BamH*I-*Xba*I fragment of the *ddl* gene into pAM401 digested with the same enzymes. To construct plasmid pJW3, the DNA fragment of the *ddl* gene was ligated downstream from the *vanH* promoter in plasmid pJW1 after digesting with *BamH*I-*Xba*I. Competent *E. coli* DH5α was used in the cloning the recombinant vectors. Transformed *E. coli* was selected by culture on a LB agar plate containing chloramphenicol 20 μg/mL. Recombinant plasmids were

prepared using a commercial kit (QIA prep® Spin Miniprep kit (250), Qiagen, USA), and inserts were identified by nucleotide sequencing with the identical set of primers used for the amplification.

3. Electrotransformation of *E. faecalis* with recombinant plasmids

A. Production of an electrocompetent cell

Cells from a single colony of *E. faecalis* were inoculated with 5 mL of M17 medium supplemented with vancomycin at 5 μg/mL. The culture was incubated overnight at 37 ℃ without aeration. The medium with 1 mL of the overnight *E. faecalis* culture was inoculated with 100 mL of SGM17 (37.25 g/L M17 broth, 0.5 M sucrose, 2% glycine, and distilled water were mixed and autoclaved) supplemented with vancomycin 5 μg/mL. It was incubated for 18 h at 37 ℃ without aeration. Cells were collected at 1000 × g for 10 minutes at a temperature of 4 ℃, and washed with 1 volume of ice cold electroporation buffer (0.5 M sucrose and 10% glycerol). The washed cells were then centrifuged at 1,000 × g for 10 minutes. Cells were resuspended in electroporation buffer using a minimum volume to recover the cells. The resuspended cells were divided into 40 μL aliquots in sterile microcentrifuge tubes and stored at -70 ℃.

B. Electrotransformation with recombinant plasmids

Electrotransformation of the competent *E. faecalis* with pJW1, pJW2 and pJW3 was accomplished with a Bio-Rad Gene Pulser. The settings used were 2.50 kV, 200 Ω, and 25 μF. Transformed *E. faecalis* was selected by culture on a brain heart infusion agar plate containing chloramphenicol 5 μg/mL and vancomycin 6 μg/mL.

4. Measurement of the minimum inhibitory concentration (MIC) of vancomycin

The broth dilution method was used to determine the vancomycin MIC for the competent and recombinant *E. faecalis*. In a 16 well plate, brain heart infusion broth supplemented with 1024 μg/mL, 512 μg/mL, 256 μg/mL, 128 μg/mL, 64 μg/mL, 32 μg/mL, 16 μg/mL, 8 μg/mL, 4 μg/mL, 2 μg/mL and 1 μg/mL of vancomycin was inoculated into each well. An inoculum of 5×10^4 CFU/mL was suspended in each well. The plate was incubated for 24 hours at 35 ℃. The lowest concentration of vancomycin that inhibited the bacterial growth was considered to be the MIC for the respective cells. *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used as quality control strains.

5. Reverse transcriptase polymerase chain reaction(RT PCR) of *vanA* and *ddl* with the cDNA library of VRE and transformed *E. faecalis*

Total RNA of competent *E. faecalis* and *E. faecalis* transformed with pJW1, pJW2 and pJW3 was prepared using Trizol (Takara RNA PCR Kit Ver2.1, Takara Shuzo Co. Japan). The cDNA library was prepared by reverse transcription using reverse transcriptase. cDNAs of VRE and transformed *E. faecalis* were used as template for the amplification of the *vanA* and *ddl* genes. A 1032 bp fragment containing the *vanA* gene was amplified by PCR using primers corresponding to bases 6979 to 6998 and 7992 to 8010 in the *vanA* resistance gene cluster (table2. GenBank accession number M97297). A 1074 bp fragment containing the *E. faecalis ddl* gene was amplified by PCR using primers corresponding to bases 33 to 52 and 1060 to 1079 in the *E. faecalis* putative *ddl* gene (table 2. GenBank accession number U00457).

The same volumes of the RT PCR products of the *vanA* and *ddl* genes of VRE and of recombinant *E. faecalis* were loaded into 0.8% agarose gel stained by ethidium bromide.

Table 2. RT PCR primers for *vanA* and *ddl* gene expression

5′-3′ DNA sequence	Use	Coordinate
atgaatagaataaaagttgc	*vanA* sense	6979-6998[1]
tcacccctttaacgctaat	*vanA* antisense	7992-8010[1]
gtggatccttgaagattattttgttgta	putative *ddl* sense	33-52[2]
actctagattatttaaaacgattcaaagct	putative *ddl* antisense	1060-1079[2]

[1] Coordinates are based on the published Tn1546 sequence (GenBank accession number M97297).

[2] Corresponds to the published sequence of the enterococci putative *ddl* gene (GenBank accession number U00457)

Nucleotide sequences that are underlined represent modifications that were made to generate the cohesive ends used for cloning.

III. Results

1. Vancomycin MIC for VRE and *E. faecalis* transformed with recombinant plasmids

The vancomycin MIC for competent *E. faecalis* was 1024 μg/mL, for *E. faecalis* transformed with pJW1 and pJW3 this was reduced to 256 μg/mL, and the vancomycin MIC for *E. faecalis* transformed with pJW2 was reduced to a smaller extent from 1024 to 512 μg/mL (table 3).

Table 3. MIC results using the broth dilution method

Strain	Vancomycin MIC (μg/mL)
competent *E. faecalis*	1024
E. faecalis -pJW1	256
E. faecalis -pJW2	512
E. faecalis -pJW3	256

S. aureus ATCC 29213 and *E. faecalis* ATCC 29212 were used as quality control strains.

2. RT PCR of the *vanA* and *ddl* genes of VRE and transformed *E. faecalis*

The *vanA* expressions in competent *E. faecalis* and *E. faecalis* transformed with pJW1, pJW2, and pJW3 were evaluated by RT PCR. The *vanA* gene of VRE and *E. faecalis* transformed with pJW1, pJW2, and

pJW3 were similarly expressed (figure 2).

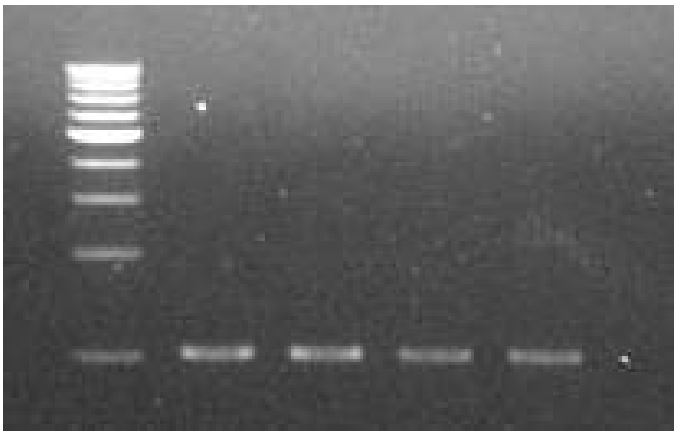

Figure 2. Reverse transcriptase PCR for detection of the expression of *vanA* mRNA.

a. VRE b. VRE transformed with pJW1 c. VRE transformed with pJW2 d. VRE transformed with pJW3

The *ddl* expressions in competent *E. faecalis* and *E. faecalis* transformed with pJW1, pJW2, and pJW3 were evaluated by RT PCR. The *ddl* gene of VRE and *E. faecalis* transformed with pJW1, pJW2, and pJW3 were found to be similarly expressed (figure.3).

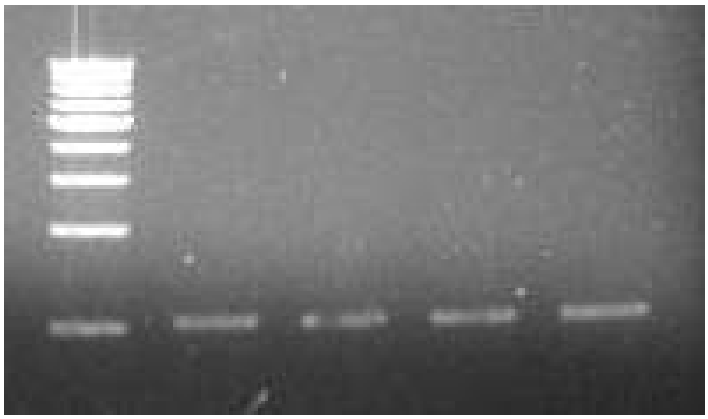

Figure 3. Reverse transcriptase PCR for detection of the expression of *ddl* mRNA. a. VRE b. VRE transformed with pJW1 c. VRE transformed with pJW2 d. VRE transformed with pJW3

IV. Discussion

Some attempts to inhibit pathogens that are resistant to antimicrobial agents by gene based strategies have been studied[18,19,21]. One such study presented a model for reversing high-level vancomycin resistance with anti-drug resistance determinant gene transfer in vancomycin resistant enterococci[11]. In this study, Torres Viera *et al.* demonstrated the restoration of vancomycin susceptibility in *E. faecalis* by antiresistance determinant gene transfer. In the study, recombinant shuttle vector containing a *vanH* promoter-*vanA* antisense gene cassette fully restored vancomycin susceptibility. They suggested that the introduction of an exogenous *vanH* promoter could sequester phosphorylated *vanR* from the native *vanH* promoter, thereby interfering with the activation of *vanH, -A,* and *-X* expression, and suggested that the expression of *vanA* antisense RNA could induce the degradation of *vanHAX* RNA. In the study, recombinant shuttle vectors containing the *vanH* promoter effected a partial restoration of vancomycin susceptibility.

Cell wall peptidoglycan is essential to most bacteria, and provides the tensile strength that provides resistance to osmotic lysis. This rigid framework is composed of repeated disaccharide units

(N-acetylglucosamine-(β-1,4)-N-acetylmuramic acid) to which the pentapeptides are attached. The majority of the pentapeptide chains (L-alanine-γ-D-glutamate-(a diamino acid)-D-alanine-D-alanine) are cross-linked by amide bonds between the D-alanine of one peptide chain and the free amino group of the diamino acid of another, either directly or through an interpeptide bridge. Synthesis of the basic units in the cytosol starts with formation of UDP-N-acetylmuramic acid, to which the first three amino acids are sequentially added. The two C-terminal D-alanyl-D-alanine residues are synthesized as a dipeptide by a D-alanine-D-alanine ligase and are added to UDP-N-acetylmuramyl-tripeptide[22]. The glycopeptides, vancomycin and teicoplanin, sterically block the access of transglycosylase and transpeptidases to their substrates by binding to the C-terminal D-alanine residues[23,24].

In VRE the *vanHAX* genes encode a new pathway of enzymes that reduces pyruvate to D-lactate (*vanH*), and combine D-alanine and D-lactate to produce D-alanyl-D-lactate (*vanA*), and then hydrolyze the normal metabolite D-alanyl-D-alanine while sparing D-alanyl-D-lactate (*vanX*)[8,9]. In VRE only the D-alanyl-D-lactate accumulates and this serves as a substrate to be elongated and presented at the termini of the peptidoglycan strands. The reprogramming of the peptidoglycan to end in D-alanyl-D-lactate

rather than the normal D-alanyl-D-alanine has no effect on the crosslinking efficiency carried out by the transpeptidating PBPs, but the switch from the D, D-dipeptide terminus to D, D-depsipeptide terminus lowers the binding affinity of vancomycin in 1,000-fold[8,10], and enables the VRE to grow at 1,000-fold-higher levels of antibiotics.

Evers *et al.* reported the sequence of *ddl* gene coding *E. faecalis* D-alanine-D-alanine ligase related protein[25]. In the present study, we obtained the *ddl* gene by PCR using primers corresponding to the sequence of the putative *ddl* gene.

In the present study, we cloned the putative *ddl* gene downstream of the *vanH* promoter. We hypothesized that the *vanH* promoter would sequestrate phosphorylated *vanR* and that the *ddl* gene would be expressed dominant to *vanA* in condition of existence of vancomycin. Therefore, we expected that the vancomycin resistance of VRE transformed with recombinant plasmid would be restored by the expression of *ddl* instead of *vanA*. However, transformation of the recombinant plasmid restored the vancomycin resistance of *E. faecalis* partially, and *ddl* did not contribute to the restoration of vancomycin resistance. RT PCR of *vanA* and *ddl* revealed that *vanA* and *ddl* were expressed in vancomycin resistant *E. faecalis*. Therefore, we believe that another mechanism is involved in the partial restoration

of vancomycin susceptibility in *E. faecalis* transformed with pJW1, and that another gene expression such as that of *vanX* or *vanY* is also important in vancomycin resistance.

This study presents a model for reversing high-level vancomycin resistance with gene transformation in enterococci. Several clinically applicable prokaryotic gene delivery modalities could be employed in future studies. These include enterococcal bacteriophage, conjugative plasmids-transposons, or modified or liposomally packaged oligonucleotides. In the future, the development of an effective gene delivery system will contribute to the design of new modalities that may overcome the limitations of antimicrobial therapy.

V. Conclusion

This study presents a model for altering high-level vancomycin resistance with gene transformation in enterococci. The *vanA* and *ddl* gene were expressed in vancomycin resistant *E. faecalis*. In the future, the development of an effective gene delivery system will undoubtedly contribute to the design of new modalities that may overcome the limitations of antimicrobial therapy.

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국문요약

vanH 프로모터 및 *ddl* 유전자 전달을 통한 반코마이신 내성 장구균의 반코마이신 감수성 변화

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반코마이신 내성 장구균은 반코마이신이 결합하는 세포벽 합성의 중간 물질인 D-alanyl-D-alanine 이중펩타이드를 반코마이신과의 결합력이 현저히 저하된 D-alanyl-D-lactate 등의 펩타이드로 대치하여 반코마이신이 결합하지 못하게 하므로써 내성을 나타내게 된다. VanA 표현형 반코마이신 내성 장구균의 반코마이신 내성을 매개하는 Transposon 1546 내의 각 유전자 중 *vanH*는 D-alanyl-D-alanine 이중펩타이드를 분해하고, *vanA*는 D-alanyl-D-lactate 연결효소로 전사되어 D-alanyl-D-lactate 펩타이드를 형성한다. 본 연구에서는 유전자 전달을 통해 VanA 표현형 반코마이신 내성 장구균의 반코마이신 감수성을 실험실 내에서 회복시키고자 하였다.

VanA 표현형 *Enterococcus faecalis* 의 DNA를 주형으로 하여 중합효소 연쇄반응을 이용하여 *vanH* 프로모터를 포함하는 445 bp의 DNA 절편과

*E. faecalis*의 *ddl* 유전자에 해당하는 1074 bp의 DNA 절편을 증폭시켰다. v*anH* 프로모터를 포함하는 DNA 절편과 pAM401을 각각 제한효소 *Sal*I과 *Sph*I으로 절단하여 T4 DNA 연결효소로 연결시켜 *vanH* 프로모터를 클로닝한 플라스미드 pJW1을 구성하였다. *ddl* 유전자에 해당하는 DNA 절편과 pAM401을 각각 제한효소 *BamH*I과 *Xba*I으로 절단하여 T4 DNA 연결효소로 연결시켜 *ddl* 유전자를 클로닝한 플라스미드 pJW2를 구성하였다. *ddl* 유전자를 플라스미드 pJW1의 vanH 프로모터 3‘ 후방에 클로닝하여 플라스미드 pJW3를 구성하였다. VanA 표현형 반코마이신 내성 장구균을 competent cell로 변형시켜 플라스미드 pJW1, pJW2, pJW3을 전기 형질 전환시켰다. Competent *E. faecalis*와 pJW1, pJW2, pJW3를 형질 전환시킨 재조합 *E. faecalis*에 대한 반코마이신 최소 억제 농도를 한천 희석법을 이용하여 측정하였다. Competent *E. faecalis*와 pJW1, pJW2, pJW3를 형질 전환시킨 재조합 *E. faecalis*에서 *vanA* 유전자와 *ddl* 유전자의 발현 여부를 역전사효소 중합효소 연쇄반응을 이용하여 평가하였다.

Competent *E. faecalis*에 대한 반코마이신 최소 억제 농도는 1024 μg/mL 이었다. 플라스미드 pJW1과 pJW3를 형질 전환시킨 *E. faecalis*에 대한 반코마이신 최소 억제 농도는 256 μg/mL 로 감소되었으나 플라스미드 pJW2를 형질 전환시킨 *E. faecalis*에 대한 반코마이신 최소 억제 농도는 512 μg/mL 이었다. *vanA* 와 *ddl* 유전자는 competent *E. faecalis*와 형질 전환시킨 *E. faecalis*에서 모두 발현되었다.

본 연구는 반코마이신 내성 장구균에서 반코마이신에 대한 내성이 유전자 전달을 통해 부분적으로 회복될 수 있다는 것을 실험적으로 증명하였다.

반코마이신 내성 장구균에서 *vanA*와 *ddl* 유전자가 모두 발현되고 있었다. 향후, 효과적인 유전자 전달 체계의 발전이 항생제 내성 균주에서 항생제 치료의 한계를 극복하기 위한 새로운 방법의 개발에 기여할 것으로 생각된다.

핵심 단어: 장구균, 반코마이신, 반코마이신 내성 장구균, 형질 전환, 유전자 전달