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Examination of Putative β-Lactamase Genes in the Soil Bacterium, *Brachybacterium faecium*

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Abstract

The importance of characterizing antibiotic resistance genes in environmental microorganisms is becoming apparent as recent research highlights the ability of environmental microorganisms to act as antibiotic resistance gene reservoirs for clinical pathogens. The genome of the soil bacterium, *Brachybacterium faecium* type strain Schefferle 6-10^{T}, was sequenced in 2009, after which the genome underwent automated annotation. An examination of the annotated genome suggested the presence of eleven putative β-lactamase genes. β-lactamase is an enzyme that provides resistance to β-lactam antibiotics. Six of the eleven putative β-lactamase genes were identified via manual annotation—using Integrated Microbial Genomes Annotation Collaborative Toolkit software—as candidates for laboratory analysis for β-lactamase activity. The ability of the putative β-lactamase gene products to provide β-lactam resistance to *Escherichia coli*—a model bacterium that lacks intrinsic β-lactam resistance—was examined via PCR amplification of the putative β-lactamase genes in *B. faecium*, transformation of competent *E. coli* cells with expression vectors containing the six putative β-lactamase genes and subsequent culture dependent determination of the minimum inhibitory concentration of β-lactam antibiotics. Characterization of viable β-lactamase genes within *B. faecium* may provide evidence for the potential of environmental microorganisms to act as antibiotic resistance gene reservoirs and may enable researchers to minimize antibiotic resistance via proper utilization of current antibiotics and strategic synthesis of new antibiotics with low environmental resistance gene frequencies.

1. Introduction

Antibiotic resistance genes are those genes that when expressed provide resistance to particular classes of antibiotics[1,2]. Resistance to antibiotic compounds by pathogenic bacteria is a clinical concern. Most antibiotic resistance genes originate in environmental microorganisms where they afford protection against self-produced antibiotics, provide resistance to those produced by other bacteria and fungi or provide a metabolic function, with antibiotic resistance as a secondary function[1,2,3,4,5,6,7,8]. Environmental microorganisms can act as a reservoir of antibiotic resistance genes for clinically important pathogens[4,6,8,9,10]. Horizontal gene transfer mechanisms—conjugation, transformation and transduction—allow microorganisms to transfer genetic material within and between species[8,11,12,13]. Most antibiotic resistance genes found in pathogens are acquired via horizontal gene transfer, and recent research suggests that many of those are transferred from environmental microorganisms[2,3,4,6,8,10].

β-lactam antibiotics are among the most extensively used antibiotics[4,14]. β-lactams act as a bactericide via the inhibition of penicillin-binding proteins (PBPs), which normally function as transpeptidase enzymes in bacterial cell wall formation[4,12,15,16]. The most common form of resistance to β-lactam antibiotics is through the enzymatic inactivation of the β-lactams via β-lactamases[1,17]. β-lactamases are classified into four groups based upon amino acid structural motifs. Classes A, C and D are active site serine β-lactamases and class B are metalloenzymes that utilize

active-site zinc ions[14,16,18]. Active site serine β-lactamases provide microorganisms with the ability to hydrolyze β-lactams via a nucleophilic attack—the hydroxyl located in the side chain of serine acts as a nucleophile that attacks the β-lactam amide bond to form an acyl intermediate, which then undergoes deacylation by a water molecule to form a carboxylic acid, effectively inactivating the β-lactam[16,18]. Active site serine β-lactamases evolved from PBPs via an active site mutation which enables the entry of a water molecule needed in the hydrolysis of β-lactams[19].

The genome of the soil bacterium, *Brachybacterium faecium* type strain Schefferle 6-10^T, was sequenced in 2009 by Lapidus et al., after which the genome underwent automated annotation[20,21]. Automated genome annotation utilizes algorithms to locate putative genes and to determine potential gene products via homologous nucleotide and amino acid sequence comparisons. An examination of the annotated genome suggested the presence of eleven putative β-lactamase genes. Six of the eleven putative β-lactamase genes were identified via manual annotation—using Integrated Microbial Genomes Annotation Collaborative Toolkit software—as potential active site serine β-lactamases and as genes of interest in further laboratory characterization.

The ability of the six putative β-lactamase genes to provide resistance to β-lactam antibiotics will be determined by cloning the genes into a laboratory strain of *Escherichia coli*, a model organism which lacks intrinsic resistance to β-lactams, and then subsequent cultivation of the transgenic *E. coli* an increasing concentrations of β-lactams. It is necessary to express the putative genes in a model organism to determine the ability of each gene to provide resistance. Characterization of potential antibiotic resistance genes in environmental microorganisms may enable researchers to minimize clinical antibiotic resistance.

2. Methods

2.1. Gene Selection

The genome sequence of *Brachybacterium faecium* type strain Schefferle 6-10^T—sequenced in 2009 by Lapidus et al.—underwent automated computer annotation[20,21]. The automatically annotated genome was examined for the presence of putative antibiotic resistance genes. Eleven putative β-lactamase genes—one class A, four class B and six class C—were located within the genome of *B. faecium*. The validity of the automated annotations of the eleven genes were examined via manual annotation through the use of the Integrated Microbial Genome Annotation Collaborative Toolkit (IMG-ACT)[21]. Sequence based similarity data—obtained using Basic Local Alignment Search Tool (BLAST) analysis and the Conserved Domain Database (CDD)—and structure based evidence from databases of manually annotated proteins and protein families—TIGRFAMs, PFAM and Protein Data Bank BLAST—were the primary IMG-ACT tools used to analyze the eleven putative genes[21,22]. The putative gene product sequences were compared to known β-lactamase protein sequences in the literature to locate the presence of β-lactamase amino acid motifs[16,23,24]. Manual annotation via IMG-ACT software and a literature review of β-lactamase enzymatic motifs enabled the identification of six of the eleven putative β-lactamase genes as candidates for laboratory analysis for β-lactamase activity.

2.2. Initial Minimum Inhibitory Concentration Tests

Penicillin G and ampicillin were selected for Minimum Inhibitory Concentration (MIC) tests based upon the potential resistance provided by the six putative β-lactamase genes[14,17,18,23,25]. MIC tests were performed initially to examine intrinsic β-lactam resistance in both *Brachybacterium faecium* (Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures type strain Schefferle 6-10^T) and *Escherichia coli* (New England Biolabs (NEB) 5-alpha High Efficiency Competent *Escherichia coli*). *B. faecium* was cultured in Tryptic Soy Yeast Extract (TSYE) and grown in a dark incubator at 30°C for three days until the culture was moderately turbid (measured as having an absorbance of .039 A at a wavelength of 625 nm via the Nanodrop 1000 spectrophotometer)[12,19,26,27]. *E. coli* was cultured in Luria-Bertani (LB) and grown in a dark incubator at 30°C for three days until the culture was moderately turbid (measured as having an absorbance of .051 A at a wavelength of 625 nm via the Nanodrop 1000 spectrophotometer)[12,26,27]. The MIC of penicillin G and ampicillin was determined for *B. faecium* and *E. coli* via a series of broths—TSYE was used for *B. faecium* and LB was used for *E.* coli—with increasing concentrations of β-lactam antibiotics (Tables 1). The MIC was determined to be the lowest concentration of antibiotic that the organism was unable to grow in (identified by the lack of any visible growth)[26,27].

Table 1. penicillin G and ampicillin MIC scheme

Concentration of β-lactam antibiotic	Volume TSYE/LB	Volume of 50.0 ug/mL stock β-lactam	Volume of cell culture
0 μg/ml	10.0 ml	0 μl	100 μl
10 μg/ml	10.0 ml	2.00 μl	100 μl
25 μg/ml	10.0 ml	4.99 μl	100 μl
50 μg/ml	10.0 ml	9.98 μl	100 μl
100 μg/ml	10.0 ml	20.0 μl	100 μl
150 μg/ml	10.0 ml	30.0 μl	100 μl
200 μg/ml	10.0 ml	39.9 μl	100 μl
0 μg/ml	10.0 ml	0 μl	0 μl

2.3. Oligonucleotide Design

Forward and reverse oligonucleotides were designed for use in PCR amplification of each of the six putative β-lactamase genes (Table 2). The primers were designed so that the amplified PCR products would include two restriction sites, an NdeI site (CA▼TATG) located at the 5' end of the amplified gene and an Spe I site (TGATC▼A) located at the 3' end of the amplified gene. (The restriction sites enabled the insertion of the amplified genes into the multi-cloning sites of the pCA21a expression vectors (Expression Technologies Inc.)). Primer BLAST (National Center for Biotechnology Information) was used to analyze the oligonucleotides to determine the potential for mis-priming and secondary structure formation[28].

Table 2. oligonucleotides (synthesized by Eurofins MWG Operon) used in amplification of putative β-lactamase genes

IMG-ACT Gene ID No.	Forward oligonucleotide, 5' - 3'	Reverse oligonucleotide, 5' - 3'
644996478	AGCTCCATATGATGAC-GCTTCCCACCGCTGCCG	GACTTACTAGTTCAGGCCGTGA-GATCGGGGAGG
644996341	AGCTCCATATGATGCCCTCAG ACATCGCCGTGC	GACTTACTAGTTCAGAGCAGCGGCAG-CAGCTC
644996411	AGCTCCATATGATGCCTG-CACCCCGCCGCTCCG	GACTTACTAGTCTAC-GCCTTCGCCTGACCTC
644998732	AGCTCCATATGATGGTCAC-GACTGCGGGGTCGG	GACTTACTAGTTCATCGCGCCTCGG-CACCGC
644998731	AGCTCCATATGATGAC-CGCGCTGGACCTGCTC	GACTTACTAG-TTCAGCCCCCGTGCTCCTCGAGCAC
644999068	AGCTCCATATGATGCCCGCCCC GACCTCCGACC	GACTTACTAG-TTCATGGCGCGGTCACCCGCTCG

2.4. DNA Isolation

Brachybacterium faecium (Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures type strain Schefferle 6-10^T) was cultured in TSYE broth. The culture was plated on a TSYE agar plate via a sterile loop to verify the purity of the broth culture. DNA was extracted from the broth culture using MP Biomedicals Fast Prep DNA Spin Kit with lysing matrix B. MP Biomedicals Fast Prep DNA Spin Kit protocol was followed for genomic

DNA isolation. The concentration and purity (260/230 ratio and 260/280 ratio) of the isolated DNA was measured using the Nanodrop 1000 spectrophotometer at a wavelength of 230 nm.

2.5. PCR

The six putative β-lactamase genes underwent amplification via PCR using an MJ mini thermal cycler (Bio Rad). A PCR master mix of 156 µl distilled water, 60.0 µl 5x NEB Phusion GC buffer, 6.0 µl 10 mM dNTPs, 30.0 µl template *B. faecium* DNA, 15.0 µl DMSO and 3.0 µl NEB Phusion DNA polymerase was prepared, with all components kept on ice (NEB Phusion DNA polymerase was added to the master mix last). 2.5 µl of the 10 mM forward oligonucleotide and 2.5 µl of 10 mM reverse oligonucleotide specific for the gene to be amplified was added to each reaction tube. A phage lambda control PCR reaction was prepared with 34 µl distilled water, 10.0 µl 5x NEB Phusion HF buffer, 1.0 µl 10 mM dNTPs, 2.5 µl of phage lambda NEB primer mix, 2.0 µl of NEB phage lambda template DNA and 0.5 µl NEB Phusion DNA polymerase. After the addition of the reaction components, the PCR reaction tubes were gently tapped down and inserted into the 98°C preheated thermal cycler. The following two-step PCR cycling procedure was utilized: an initial denaturation at 98°C for 50 seconds, 30 cycles of denaturation at 98°C for 10 seconds and annealing and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 10 minutes. The samples were held at 4°C. Upon removal from the thermal cycler the samples were stored at -20°C

The PCR products were analyzed by gel electrophoresis in a 1.5% agarose gel (prepared with 1.5% DNA grade agarose and 0.5% TBE buffer). Two DNA ladders (low scale Fisher 2000 bp ladder and NEB 23,000 bp ladder) were analyzed concurrently with the PCR products to enable the determination of the size of the PCR products for comparison with the known size of the genes.

MP Biomedicals Gene Clean Turbo kit was utilized to clean the PCR product via column isolation of the amplified genes following the MP Biomedicals protocol. The DNA concentration and purity of the samples were analyzed using the Nanodrop 1000 spectrophotometer at a wavelength of 230 nm.

2.6. Restriction Digest

The PCR products and pCA21a expression vectors (Expression Technologies Inc.) underwent a double restriction digest with NdeI and SpeI at 37°C for 1 hour. The PCR product digest reaction contained 40 µl of PCR product, 3.5 µl of distilled water, 5.0 µl Cutsmart buffer (NEB), 0.5 µl NdeI (NEB) and 1.0 µl SpeI (NEB). The expression vector digest reaction contained 2.0 µl pCA21a expression vector (Expression Technologies Inc.), 41.5 µl distilled water, 5 µl Cutsmart buffer (NEB), 0.5 µl NdeI (NEB) and 1.0 µl SpeI (NEB). The restriction enzymes were heat inactivated at 80°C for 20 minutes post digestion.

MP Biomedicals Gene Clean Turbo kit was utilized to clean the PCR product via column isolation of the amplified genes following the MP Biomedicals protocol. The DNA concentration and purity of the samples were analyzed using the Nanodrop 1000 spectrophotometer at a wavelength of 230 nm.

2.7. Ligation and Transformation

Prior to ligation the digested vector was treated with Calf Intestinal Phosphatase (CIP) via the addition of 1 µl of CIP and 3.3. µl Cutsmart buffer (NEB) to 30 µl of digested vector. MP Biomedicals Gene Clean Turbo kit was used to remove CIP prior to ligation. Instant Sticky-end Ligase Master Mix (NEB) was utilized to ligate the digested PCR products with the digested pCA21a expression vector (Expression Technologies Inc.) following the ligation protocol provided by NEB. The concentration of insert DNA to vector DNA was in a molar ratio of 1:1. The ligation reactions immediately underwent transformation following the high efficiency transformation protocol for NEB 5-alpha High Efficiency Competent *E. coli*, with an alteration in the protocol from a 60 minute shaking incubation at 37°C to a 100 minute shaking incubation at 37°C (due to the high stress created by the use of chloramphenicol to select for transformants containing the selectable marker, the chloramphenicol resistance gene, in the pCA21a expression vector). Two controls—a vector that underwent restriction digestion and ligation and an undigested vector—underwent transformation along with the six samples. The transfomation cultures (150 µl) were plated on LB agar containing 33 µg/ml chloramphenicol. The plates were incubated for 24 hours at 37°C in the dark. Transformants were selected using sterile toothpicks and cultured in LB broth containing 33 µg/ml chloramphenicol to ensure maintenance of the expression vector. The transformant cultures were placed in a 37°C, 230 rpm shaking incubator for 16 hours.

Vector DNA was isolated from the transformed cultures via the Qiagen Plasmid Isolation Kit, following the Qiagen protocol. The isolated vectors underwent a double restriction digest with NdeI and SpeI following the protocol given in section 2.6. The digested vectors were visualized via gel electrophoresis to ensure that the transformants contain both vector and insert DNA of the appropriate size.

2.8. Initial Induction and Minimum Inhibitory Concentration Test

The transformant cultures which were shown to contain inserts— gene 644996478, gene 644996341 and gene 644998731—were grown in 2 ml of LB containing 33 μg/ml chloramphenicol for 12 hours in a 37 ºC, 230 rpm shaking incubator to prepare for induction. The cultures were diluted 1:50 using LB containing 33 μg/ml chloramphenicol and were grown for 3 hours in a 37 ºC, 230 rpm shaking incubator. The 2 ml, incubated cultures were halved and 1 ml of LB containing 33 μg/ml chloramphenicol, 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) and 0-200 μg/ml of ampicillin was added to the 1 ml cultures to undergo induction concurrently with the MIC test for ampicillin. Non-induced cultures were used as a control via the addition of 1 ml of LB containing 33 μg/ml choramphenicol and 1-200 μg/ml of ampicillin to the remaining 1 ml cultures. The cultures were returned to the shaking incubator for 4 hours. Upon removal the cultures were visually examined to compare the turbidity between the induced samples and controls. Further induction and MIC tests are needed to verify any initial results using spectrophotometric analysis.

3. Results and Discussion

3.1. Manual Annotation of *B. faecium* Identifies Six Potential β-Lactamase Genes

Manual annotation—via IMG-ACT—and a literature review of β-lactamase enzymatic motifs enabled the identification of six of the eleven putative β-lactamase genes as candidates for laboratory analysis for β-lactamase activity. The sequence and structure based similarity data suggest that IMG-ACT Gene Number: 644996478 is a class A, active site serine β-lactamase, 644996341 is a penicillin-binding protein (PBP), an fmtA protein—a specialized PBP that undergoes heightened expression under β-lactam-induced cell wall stress[29]—or a class C, active site serine β-lactamase, 644996411 is a PBP, an fmtA protein, or a class C, active site serine β-lactamase, 644998732 is a PBP or an active site serine β-lactamase, 644998731 is a PBP or a class C, active site serine β-lactamase, and 644999068 is an active site serine β-lactamase or a transpeptidase (Table 3, Table 4)[17,22,23,30,]. The manual annotation suggests that the six putative β-lactamase genes are β-lactamases or PBPs.

Table 3. results from the NCBI BLAST analysis via IMG-ACT (query coverage is the percent of the query sequence that overlaps the subject sequence, maximum identity is the percent similarity between the query and subject sequences, E-value is the number of hits that are expected due to chance)

IMG-ACT Gene ID Number	Top BLAST hits (gene product, organism)	query coverage	max ID	E-value
644996478	β-lactamase class A, *Brachybacterium paraconglomeratum*	100%	66%	$7e^{-110}$
	β-lactamase class A *Brachybacterium squillarum*	99%	57%	$6e^{-83}$
	β-lactamase class A *Isoptericola veriabilis*	91%	43%	$3e^{-24}$
644996341	Penicillin-binding protein, β-lactamase class C *Brachybacterium paraconglomeratum*	99%	82%	$3e^{-175}$
	Penicillin-binding protein, β-lactamase class C *Brachybacterium squillarum*	98%	72%	$2e^{-139}$
	Penicillin-binding protein, β-lactamase class C *Haloplasma contractile*	96%	38%	$4e^{-69}$
644996411	Penicillin-binding protein, β-lactamase class C *Brachybacterium paraconglomeratum*	94%	79%	$3e^{-68}$
	Penicillin-binding protein, β-lactamase class C *Brachybacterium squillarum*	99%	64%	$2e^{-52}$
	β-lactamase *Leifsonia rubra*	96%	44%	$1e^{-22}$
644998732	Penicillin-binding protein *Brachybacterium paraconglomeratum*	93%	78%	$2e^{-148}$
	Penicillin-binding protein *Brachybacterium squillarum*	90%	80%	$1e^{-97}$
	Penicillin-binding protein, β-lactamase *Actinomyces* sp.	87%	58%	$3e^{-97}$
644998731	Penicillin-binding protein, β-lactamase class C *Brachybacterium paraconglomeratum*	99%	79%	$3e^{-143}$
	β-lactamase *Brachybacterium phenoliresistens*	99%	67%	$4e^{-119}$
	β-lactamase *Mycobacterium thermoresistibile*	98%	50%	$8e^{-76}$
644999068	β-lactamase *Brachybacterium phenoliresistens*	97%	60%	0.0
	β-lactamase *Beutenbergia cavernea*	62%	44%	$3e^{-73}$
	β-lactamase *Kribbella flavida*	83%	42%	$4e^{-70}$

Table 4. top sequence and structure based similarity hits determined using IMG-ACT—Conserved Domain Database (CDD), TIGRFAMs, PFAM, Protein Data Bank (PDP) BLAST—and corresponding E-value (the number of hits that are expected due to chance)

IMG-ACT Gene ID Number	CDD protein family hits	TIGRFAM protein family hits	PFAM protein domain hits	PDP BLAST protein hits
644996478	β-lactamase 2 ($2.1e^{-17}$)	PF00144: β-lactamase (.13)	PF13354: β-lactamase 2 ($1.3e^{-31}$)	4EWF: β-lactamase from *Sphaerobacter thermophilus* ($1.85e^{-4}$)
644996341	β-lactamase ($7.9e^{-31}$) AmpC ($3.4e^{-29}$)	PF00144: β-lactamase ($2.7e^{-31}$)	PF00144: β-lactamase ($1.8e^{-35}$)	4GDN: Fmt-A-like protein ($1.4e^{-8}$) 2QMI: octameric PBP homologue from *Pyrococcus abyssi* ($1.7e^{-8}$)
644996411	β-lactamase ($5.9e^{-46}$) AmpC ($2.7e^{-36}$)	PF00144: β-lactamase ($2.6e^{-53}$)	PF00144: β-lactamase ($1.9e^{-55}$)	2QMI: octameric PBP homologue from *Pyrococcus abyssi* ($7.1e^{-23}$) 4GDN: FmtA-like protein ($2.58e^{-21}$)
644998732	β-lactamase ($3.2e^{-26}$) AmpC ($8.7e^{-20}$)	PF00144: β-lactamase ($3.2e^{-25}$)	PF00144: β-lactamase ($3.2e^{-30}$)	3I7J:β-lactamase from *Mycobacterium bovis* ($9.7e^{-62}$)
644998731	β-lactamase ($3.6e^{-21}$) AmpC ($1.8e^{-15}$)	PF00144: β-lactamase ($5.3e^{-19}$)	PF00144: β-lactamase ($6.5e^{-24}$)	3I7J: β-lactamase from *Mycobacterium bovis* ($6.7e^{-56}$)
644999068	β-lactamase ($5.0e^{-29}$) AmpC ($3.6e^{-24}$)	PF00144: β-lactamase ($6.8e^{-36}$)	PF00144: β-lactamase ($3.1e^{-42}$)	1CEF:DD-peptidase from *Streptomyces* sp. ($5.6e^{-11}$)

3.2. Initial Minimum Inhibitory Concentration Tests Suggest that *B. faecium* Does Not Exhibit Resistance to Penicillin G and Ampicillin

The initial MIC tests suggest that *B. faecium* (Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures type strain Schefferle $6\text{-}10^{T)}$ does not possess resistance to penicillin G or ampicillin at concentrations at or above 10 μg/ml, determined visually by the lack of turbid growth. The initial MIC tests suggest that *E. coli* (NEB 5-alpha High Efficiency) possesses resistance when cultured up to 25 μg/ml of penicillin G and does not possess resistance to ampicillin at concentrations at or above 10 μg/ml. (Bacterial growth at or above 50 μg/ml of β-lactam antibiotics demonstrates resistance[26,27].) The lack of resistance exhibited by *B. faecium* in the initial MIC test does not necessarily suggest that the putative β-lactamase genes do not possess β-lactamase activity against penicillin G and ampicillin. Recent research suggests that some genes that provide antibiotic resistance in clinical pathogens, primarily provide a metabolic function to the native host organism, with antibiotic resistance as a secondary function or completely absent[2,3,5,7]. It has also been suggested that the expression of some β-lactamase genes is under the control of quorum sensing mechanisms, which enable heightened expression only under conditions in which the cellular population has reached a quorum[31].

3.3. Identification of Three Insert-Containing Transformants

The vectors of three transformants were identified as containing gene inserts—gene 644996478, gene 644996341 and gene 644998732—via a double restriction digest and subsequent gel electrophoresis visualization. The expected size of the linearized isolated vector is 3,256 bp. The linearized isolated vector was determined to be present in all of the gel lanes except for lanes 10 and 11 (Figure 1). Lane 5 contained the control vector which had undergone digestion, phosphatase treatment and ligation prior to transformation. The presence of a band around 7,000 bp in lane 5 suggests that some of the control vector may be in the form of a nicked vector (which moves through the gel more slowly than a linearized vector). In lane 5, the band at 1,800 bp suggests that some of the control vector may be held in a supercoiled configuration that did not undergo linearization (a supercoiled pieced of DNA will move through a gel more quickly than its linearized counterpart). The presence of control transformants and the variety of configurations displayed by the control vector suggests that some of the sample transformants may be due to vector only transformations. The expected size of gene 644996478 is 822 bp. The presence of the inserted gene was confirmed by the presence of bands around 822 bp in lanes 6 and 7 (Figure 1). The expected size of gene 644996341 is 927 bp. The presence of the inserted gene was confirmed by the presence of the band around 927 bp in lane 8 (Figure 1). The expected size of gene 644998732 is 876 bp. The presence of the inserted gene was confirmed by the presence of the band around 876 bp in lane 12 (Figure 1).

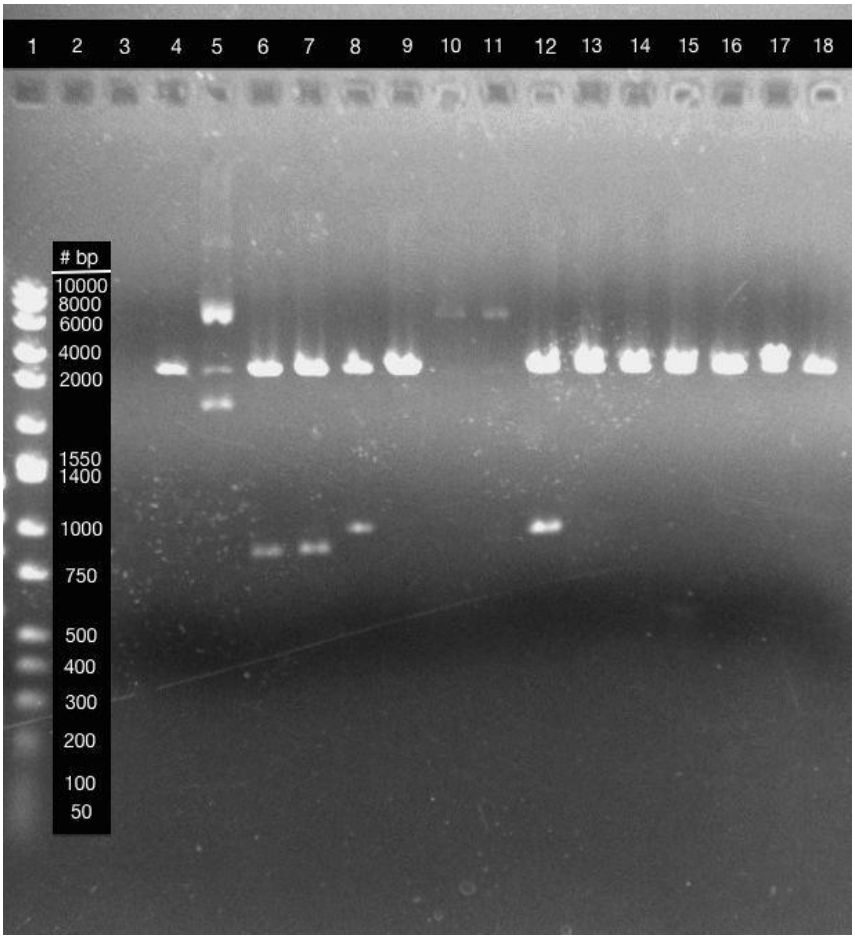

Figure 1. UV-visualized Electrophoretic Gel of Double Digested Isolated Plasmids

Figure 1. UV-visualized electrophoretic gel: Lane 1 contains Sigma-Aldrich Broad Range WideLoad DNA Ladder (50 bp-10,000 bp), Lane 3 contains a negative control, Lane 4 contains the transformed vector, Lane 5 contains the

digested, ligated and transformed vector, Lanes 6 and 7 contain transformants of gene 644996478, Lanes 8 and 9 contains transformants of gene 644996341, Lanes 10 and 11 contain transformants of gene 644996411, Lanes 12, 13 and 14 contain transformants of gene 644998732, Lanes 15 and 16 contain transformants of gene 644998731, and Lanes 17 and 18 contain transformants of gene 644999068.

3.3. Minimum Inhibitory Concentration Tests in Transgenic *E. coli* are Preliminary and Do Not Provide Evidence For or Against β-Lactamase Activity

The minimum inhibitory concentration tests in transgenic *E. coli* post induction were inconclusive due to the preliminary nature of the concurrent induction process and MIC testing that was used. The combination of induction with MIC testing needs to be further modified to achieve results.

4. Conclusion

The lack of demonstrable resistance does not negate the potential of the putative β-lactamase genes in *Brachybacterium faecium* to provide resistance to β-lactam antibiotics. Further research is necessary to characterize the putative β-lactamase genes and gene products. Manual gene annotation suggests that the genes under examination encode active site serine β-lactamases or penicillin binding proteins. The characterization of environmental microorganisms is needed to better understand the total microbial gene pool to enable researchers to determine potential genes that may enable pathogenic resistance to antimicrobial drugs[32].

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