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Transposon insertion in *Rothia dentocariosa*

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Abstract

Objective: *Rothia* spp. are emerging as important bacteria associated with oral health; among which, *Rothia dentocariosa* is one of the most common species. However, no studies have examined its properties at the genetic level. This study aimed to establish a genetic modification platform for *R. dentocariosa*.

Methods: *Rothia* spp. were isolated from the saliva samples collected from volunteers with healthy oral conditions. Subsequently, *R. dentocariosa* strains were identified by colony morphology, species-specific PCR, and 16S ribosomal RNA gene sequencing. The identified strains were transformed with plasmid pJRD215, and the most efficient strain was selected. Transposon insertion mutagenesis was performed to investigate the possibility of genetic modifications.

Results: A strain with a high transforming ability was successfully identified and named *R. dentocariosa* LX16. This strain was subjected to transposon insertion mutagenesis and screened for 5-fluoroorotic acid-resistant transposants; their insertion sites were confirmed using arbitrary primed PCR, gene-specific PCR, and Sanger sequencing.

Conclusion: This study demonstrated for the first time the successful genetic modification of *R. dentocariosa*. Studying *R. dentocariosa* at the genetic level can elucidate its role in the oral microbiome.

keywords: *Rothia dentocariosa*, genetic modification, transposon insertion mutagenesis

1. Introduction

In 1967, Georg and Brown first identified the genus *Rothia* [1]. *Rothia aerea*, *R. dentocariosa*, and *Rothia mucilaginosa* are the primary indigenous *Rothia* species found in the oral cavity of

healthy oral flora [2-5] but decrease significantly with the progression of gingivitis and periodontitis [6-11]. Huang et al. reported a strong association between deterioration of oral hygiene, progression of gingivitis, and a decrease in the abundance of *Rothia* spp. in the oral microbiota [12]. Therefore, *Rothia* spp. may play crucial roles in oral health and serve as important oral health-associated bacteria.

Rothia spp. can reduce nitrate to nitrite, which is ultimately converted into nitric oxide (NO) [13, 14]. NO exhibited antimicrobial activity against periodontal pathogens [15, 16, 17]. Furthermore, it has been experimentally demonstrated that adding nitrate to *in vitro* oral biofilm containing nitrate-reducing bacteria suppresses the decrease in pH of the culture medium, inhibits lactic acid production, and increases ammonium production [14, 18]. These findings indicate an association between nitrate-reducing bacteria and oral health. Therefore, *R. dentocariosa* has potential health benefits.

R. dentocariosa is one of the most common species among oral *Rothia* spp. [3]. Although the biochemical properties of *R. dentocariosa* have been reported [13, 19, 20], the bacteria have not been studied at the genetic level, largely due to the lack of a suitable technique for genetic manipulation of *R. dentocariosa*.

This study established a genetic modification technology platform for *R. dentocariosa*. We isolated *R. dentocariosa* strains with a high transformation efficiency from the saliva. Genetic modifications were performed by transposon insertion mutagenesis.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The strains used in this study were *R. aeria* JCM 11412, *R. dentocariosa* AIu 17931, *R. mucilaginosa* DY18, and *R. dentocariosa* strains isolated from saliva. *Rothia* spp. were grown in heart infusion broth (HIB) (Becton, Dickinson and Company, Sparks, MD, USA) or on HIB agar plates (HIA) at 37 °C for 36–48 h aerobically. Antibiotics were used at the following concentrations when indicated: kanamycin (Km) (Wako Pure Chemical Industries, Osaka, Japan), 25 µg/mL; streptomycin (Sm) (Wako Pure Chemical Industries), 25 µg/mL.

2.2. Isolation of R. dentocariosa by colony morphology

Saliva samples were collected from 3 volunteers with healthy oral conditions. Each volunteer

was instructed to allow saliva to pool in their mouth and then spit at least 3 mL of saliva into a sterile tube. The collected samples were diluted 1,000 times with phosphate-buffered saline (PBS; pH 7.2). Fifty microliters of the dilutions were plated onto oral *Rothia* species selective medium (ORSM) [21] for isolating *Rothia* spp., and the plates were then incubated at 37 °C for 48–60 h aerobically. The strains grown on ORSM were initially distinguished by colony morphology, and then colonies resembling *R. dentocariosa* were streaked twice on fresh HIA. Purified isolates were cultured in HIB and stored at –80 °C in HIB containing 10% dimethyl sulfoxide (DMSO) (Wako Pure Chemical Industries).

2.3. Identification of *R. dentocariosa* by species-specific PCR

The isolated strains were identified using a species-specific PCR designed by Tsuzukibashi et al. [21]. A single colony was suspended in 200 µL of sterile distilled water and mixed thoroughly. Twenty-one microliters of this solution were added to a PCR reaction mixture containing 1.25 units of MightyAmp DNA polymerase Ver.3 (Takara Bio, Shiga, Japan), 25 pmol of each primer, and 25 µL of 2× MightyAmp buffer Ver.3 (Takara Bio). The PCR was performed according to the manufacturer's instructions. The amplicons were analyzed by electrophoresis on a 1.5% agarose gel containing ethidium bromide under UV light.

2.4. Identification of *R. dentocariosa* by the 16S ribosomal RNA (rRNA) gene

The 16S rRNA gene was amplified by PCR using the 27F and 1492R primers [22]. Genomic DNA was extracted from each isolate using NucleoSpin Microbial DNA (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. One hundred nanograms of genomic DNA were added to a PCR reaction mixture containing 25 µL of 2× PrimeSTAR Max Premix (Takara Bio) and 25 pmol of each primer. The PCR was performed according to the manufacturer's instructions. The resulting amplicons were analyzed using gel electrophoresis, as described previously. The amplicons were purified by gel purification using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel) according to the manufacturer's instructions. Subsequently, the purified amplicons were sequenced on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA), and the resulting nucleotide sequences were analyzed using the basic local alignment search tool nucleotide (BLASTN) against the NR/NT database of the NCBI database. Additionally, we used the MEGA11 software (<http://www.megasoftware.net/>) to compare the 16S

rRNA sequences of each isolate with those of *R. aeria* JCM 11412, Lan, KCOM 1021, *R. dentocariosa* ATCC 17931, ATCC BAA-907, KCOM 3741, *R. mucilaginosa* ATCC 25296, KCOM 2049, FC6533, and *Micrococcus luteus* ATCC 15307 obtained from NCBI to construct a phylogenetic tree. *Micrococcus luteus*, closely related to *Rothia* spp. [23], served as a blank group in phylogenetic tree construction. The tree construction process utilized the neighbor-joining method (1000 bootstraps), in which the Tamura-Nei method [24] was used to calculate evolutionary distances.

2.5. Competent cells and electroporation of *pJRD215*

The isolates were grown in HIB at 37 °C with shaking at 121 rpm overnight, aerobically. The culture was diluted in 20 mL of fresh HIB to an optical density of 0.1 at 600 nm (OD₆₀₀) and the diluted solution was incubated until the culture reached an OD₆₀₀ of 0.4–0.6. The solutions were incubated on ice for 10 min. The cells were pelleted by centrifugation at 11,000 × g for 5 min at 4 °C. The cell pellet was washed thrice with 10% (v/v) cold aqueous glycerol solution: 20 mL for the first wash, 10 mL for the second wash, and 5 mL for the final wash. Subsequently, the cells were resuspended in 100 µL of 10% cold glycerol as electrocompetent cells.

A 1.5 mL tube was used to combine 50 µL of the electrocompetent cells and 100 ng of *pJRD215*, a broad host-range plasmid carrying the Km and Sm resistance genes [25], on ice. The mixture was transferred to a 0.1 cm gap sterile electroporation cuvette (BM Equipment Co., LTD, Tokyo, Japan) on ice. Electroporation was carried out at 2.5 kV, 25 µF, and 200 Ω using a Gene Pulser II system (Bio-Rad Laboratories, Richmond, CA, USA). Subsequently, the mixture was resuspended in 950 µL of HIB at room temperature, incubated at 37 °C for 1 h without shaking, aerobically, then plated on HIA with Km and Sm, and incubated at 37 °C for an additional 48 h. Transformation efficiency was determined by counting colonies grown on HIA with Km and Sm, expressed as colony-forming units per microgram of plasmid DNA (CFU/µg). The strain with the highest transformation efficiency, LX16, was used for subsequent experiments.

2.6. Transposon insertion mutagenesis

To investigate the possibility of genetic modification on the genome of *R. dentocariosa* LX16, transposon insertion mutagenesis was attempted on *R. dentocariosa* LX16 using the EZ-Tn5™ Tnp Transposome™ Kit (EZ-Tn5) (Epicentre Biotechnologies, Madison, WI, USA), incorporating

the Km resistance genes. A 1.5 mL tube was used to combine 50 μ L of electrocompetent cells and 0.5 μ L of EZ-Tn5 on ice, then the mixture was transferred to a 0.1 cm gap sterile electroporation cuvette on ice. Electroporation was carried out at 2.5 kV, 25 μ F and 200 Ω . The cells were then resuspended in 950 μ L of HIB at room temperature and incubated at 37 °C for 1 h without shaking, aerobically. To elucidate the insertion of EZ-Tn5 into the LX16 genome and the resulting phenotypic changes caused by gene disruption, screening using 5-FOA was carried out. The culture was plated on HIA with Km and 5-fluoroorotic acid (5-FOA) (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) (100 μ g/mL) and then incubated at 37 °C for 48 hours aerobically.

2.7. Determination of transposon insertion site

Genomic DNA of 5-FOA resistant transposants was extracted using NucleoSpin Microbial DNA according to the manufacturer's instructions. To confirm the insertion site of EZ-Tn5 on the genome, the arbitrary primed PCR (AP-PCR) [26] was performed on the extracted DNA. The resulting amplicons were analyzed by gel electrophoresis as described previously. The amplicons were then purified by gel purification using NucleoSpin Gel and PCR Clean-up according to the manufacturer's instructions. Subsequently, the purified amplicons were sequenced on an ABI 3730xl DNA analyzer, and the resulting nucleotide sequences were analyzed using the Basic Local Alignment Search Tool X (BLASTX) against the non-redundant (NR) protein database from the NCBI database.

2.8. PCR confirmation of transposon insertion

To confirm whether EZ-Tn5 was inserted at the location determined by AP-PCR, primers were designed to be located approximately 100 bp upstream and downstream of the transposon insertion position (Table 1). PCR was performed using PrimeSTAR Max Premix according to the manufacturer's instructions. The resulting amplicons were analyzed by gel electrophoresis as described previously.

3. Results

3.1. Isolation and identification of R. dentocariosa

Rothia spp. were successfully isolated from saliva samples using ORSM. *R. dentocariosa* appeared as smooth, circular colonies, whereas *R. aeria* and *R. mucilaginosa* exhibited highly

irregular shapes (Fig. 1). Subsequently, smooth circular colonies were streaked twice on fresh HIA, and the isolated colonies were identified using species-specific PCR based on 16S rRNA gene sequencing. Using this method, different PCR product sizes were obtained depending on the *Rothia* spp.. The expected product sizes were 143 bp for *R. aeria*, 551 bp for *R. dentocariosa*, and 400 bp for *R. mucilaginosa*. The results showed that 8 bacterial strains were identified as *R. dentocariosa*, displaying bands of expected length (Fig. 2). Eight strains were identified using 16S rRNA gene sequencing and BLASTN analysis (Table 2). The 16S rRNA gene sequences of each isolate were aligned with those of the reference strains to construct a phylogenetic tree (Fig. 3). Based on the branching pattern, the reference *Rothia* strains were divided into 3 major clades in the phylogenetic tree created by the neighbor-joining method using 16S rRNA. All 8 isolated strains were classified into the *R. dentocariosa* clade. Based on the geographical locations of the isolates, the trees displayed genetic heterogeneity and distances within the species.

3.2. Transformation efficiency of plasmid pJRD215 mediated by electroporation

We screened strains exhibiting high transformation efficiency using the broad host-range plasmid pJRD215 [25] to achieve genetic modification of *R. dentocariosa*. A strain named LX16 stood out with the transformation efficiency 7.4×10^3 CFU/ μ g. However, the remaining 7 strains of *R. dentocariosa*, apart from LX16, were incapable of genetic transformation, and their data are not presented.

3.3. Transposon insertion mutagenesis

We performed transposon insertion mutagenesis using EZ-Tn5 to explore the potential for the genetic modification of LX16. Screening using transposon antibiotic markers (Km) and 5-FOA resulted in the identification of 7 clones. These clones were named LF1–LF7.

3.4. Identification of the transposon insertion sites

AP-PCR, Sanger DNA sequencing, and BLAST analyses were performed to determine the genomic insertion sites of EZ-Tn5. The results indicated that transposons were inserted into 6 different genes. Two clones, LF4 and LF5, contained transposon insertions at different loci in the same gene (Fig. 4, Table 3). Gene-specific PCR was used to confirm the insertion sites of EZ-Tn5 in these genes. The primers were designed to be 100 bp upstream and downstream of the insertion position (Fig. 4, Table 1). The PCR product was approximately 200 bp in the wild type strain,

whereas it extended to approximately 1.4 kb in the transposants (Fig. 5).

4. Discussion

In the present study, we isolated a genetically tractable strain of *R. dentocariosa*, LX16, which exhibited high transformation efficiency. Subsequently, we successfully performed genetic modifications by transposon insertion into the strain. To our knowledge, this is the first report of genetic modification of *R. dentocariosa*.

Genomic information on 57 strains of *R. dentocariosa* was obtained by searching the NCBI database. However, no studies have investigated the genetic modifications in *R. dentocariosa*. We believe that the establishment of a technological platform for genetic modification is urgently needed. Therefore, we attempted to isolate a novel genetically tractable *R. dentocariosa* from the oral cavity.

We initially transformed the isolates with the broad-host-range plasmid, pJRD215, to screen for strains capable of genetic modification. This plasmid, derived from RSF1010 [27], has been confirmed to effectively transform many Gram-positive bacteria [28,29], particularly *Actinomyces viscosus* [25], which shares phylogenetic proximity with *R. dentocariosa* [23]. Based on this information, we hypothesized that pJRD215 can replicate in *R. dentocariosa*. Our experiment led to the identification of *R. dentocariosa* strain LX16, with a notable transformation efficiency of 7.4×10^3 CFU/ μ g. In contrast to the reported efficiency of *A. viscosus* at 2.9×10^7 CFU/ μ g [25], our findings indicated a lower efficiency. As this study examined a single condition, it is imperative to introduce variations in the culture conditions and transformation parameters to optimize the processes.

We performed transposon insertion mutagenesis of LX16 to explore its potential for genetic modifications. Given that no previous cases of transposon insertion mutagenesis in *R. dentocariosa* have been reported, we drew inspiration from a study conducted by Fujihira et al., in which EZ-Tn5 transposon insertion mutagenesis was applied to *R. mucilaginosa* [30]. Since *R. mucilaginosa* and *R. dentocariosa* belong to the *Rothia* genus, we hypothesized that EZ-Tn5 would be similarly effective against *R. dentocariosa*. Therefore, we chose EZ-Tn5 mutagenesis as the initial approach for *R. dentocariosa* genetic modification.

Following the transposon insertion mutagenesis, phenotypic screening based on 5-FOA

resistance was performed, resulting in the acquisition of 7 clones. Genomic insertion of transposon EZ-Tn5 in each clone was confirmed using AP-PCR, Sanger sequencing, BLAST analysis, and gene-specific PCR. This outcome provides evidence that trait changes can be achieved through genetic modification in *R. dentocariosa*.

5-FOA is used as a counter-selectable marker for gene deletion in many bacteria, including *Actinomycetes* such as *Streptomyces rimosus* and *Mycobacterium smegmatis* [31,32], closely related to *R. dentocariosa* [23]. Therefore, we were interested in determining whether 5-FOA could also function as a counter-selectable marker for *R. dentocariosa*. We streaked the LX16 strain on HIA medium containing 100 µg/mL of 5-FOA; the results indicated that the LX16 strain could not survive. Furthermore, investigation of *R. dentocariosa* genome using the NCBI database confirmed the presence of *pyrE* and *pyrF* genes. These observations form the basis of our study using 5-FOA as a counter-selectable marker for genetic engineering of *R. dentocariosa*. 5-FOA is an analog of orotic acid (OA). According to the KEGG website (<https://www.genome.jp/kegg/pathway.html>) data on the pyrimidine biosynthetic pathway in *R. dentocariosa*, OA is formed orotidine-5'-monophosphate (OMP) under the catalysis of the orotate phosphoribosyltransferase encoded by the *pyrE* gene. The resulting OMP is subsequently decarboxylated to uridine 5'-monophosphate (UMP) by the OMP decarboxylase encoded by the *pyrF* gene. Similarly, through the catalysis by these 2 enzymes, 5-FOA forms 5-fluoroorotidine-5'-monophosphate (5-FOMP) and is decarboxylated to cytotoxic 5-fluorouridine monophosphate (5-FUMP), killing cells upon accumulation in the cells [33]. Utilizing this characteristic as a counter-selectable marker for bacterial genetic modification allows the establishment of genetically deficient mutants [31,32].

The results of this study, in which 5-FOA resistant strains LF1-LF7 were successfully generated, indicated that EZ-Tn5 was not inserted into *pyrE* or *pyrF* genes in these resistant strains. Upon re-evaluating the formation process of 5-FUMP, we discovered that 5-phosphoribosyl-1-pyrophosphate (PRPP) also plays a role in the formation of 5-FUMP. The *pyrE* enzyme catalyzes the condensation of 5-FOA with PRPP, resulting in the formation of cytotoxic 5-FUMP under catalysis by the *pyrF* enzyme. In the LF6 strain, EZ-Tn5 was inserted into a gene encoding an NAD(P)H-dependent oxidoreductase, possibly affecting the synthesis of

PRPP [34,35], thereby preventing the conversion of 5-FOA to 5-FUMP. In bacteria, the PRPP biosynthetic pathway originates from glucose. Several NAD(P)H-dependent oxidoreductases are required for this process. Among these enzymes, 6-phosphogluconate dehydrogenase catalyzes the oxidative decarboxylation of 6-phosphogluconate to ribulose 5-phosphate. If this enzyme is deficient, then PRPP cannot be produced. The 5-FOA resistance of the LF6 strain may be due to a deficiency in 6-phosphogluconate dehydrogenase. For the LF2 and LF7 strains, EZ-Tn5 was inserted into the genes encoding the TetR and MarR family transcriptional regulators. These proteins are involved in the repression of gene transcription [36,37] and may affect the expression of genes related to 5-FOA metabolism. For LF1, EZ-Tn5 was inserted into a gene encoding the AhpC/TSA family, involved in bacterial antioxidant defense [38]. LF3 has EZ-Tn5 inserted into the gene encoding the LytR C-terminal domain-containing protein, which is involved in the assembly of gram-positive bacterial cell walls [39]. For LF4 and LF5, EZ-Tn5 was inserted into the gene encoding a pentapeptide repeat-containing protein. This protein family has more than 500 members, most of which have unclear biochemical functions [40]. Although our results showed that these proteins directly influence 5-FOA resistance in *R. dentocariosa*, the specific mechanisms by which they affect 5-FOA resistance remain unclear. Owing to the lack of a suitable technique for genetic manipulation of *R. dentocariosa*, we are currently unable to experimentally verify the functions of these EZ-Tn5 inserted genes. Therefore, to gain a deeper understanding of the complex and interactive molecular mechanisms of *R. dentocariosa*, the development of a gene deletion mutagenesis system for *R. dentocariosa* is particularly crucial.

5. Conclusion

This study successfully identified a strain, *R. dentocariosa* LX16, capable of plasmid transformation and genetic modification through transposon insertion mutagenesis. To our knowledge, this is the first report of the genetic modification of *R. dentocariosa*.

To elucidate the functions of bacterial genes, it is essential to create mutants with disrupted genes. The results of this study demonstrate the ability to analyze *R. dentocariosa* at the genetic level. In the future, we plan to develop a gene deletion mutagenesis system for *R. dentocariosa* based on the results of this study.

Ethical statement

This study was approved by the Osaka Dental University Ethics Committee (approval number 111237-0) and conducted in compliance with the relevant guidelines and regulations.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Author contribution statement

Boang Liu; performing experiments, writing of the first draft of this manuscript, completion of this manuscript, Chiho Mashimo; study design, critical revision of this manuscript, completion of this manuscript, final approval of the version to be submitted, Takayuki Nambu; critical revision of this manuscript, completion of this manuscript, Hugo Maruyama; critical revision of this manuscript, completion of this manuscript, Toshinori Okinaga; critical revision of this manuscript, completion of this manuscript, final approval of the version to be submitted.

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Fig legends

Fig. 1 Stereomicroscope image of *R. dentocariosa*, *R. mucilaginoso*, and *R. aeria* colonies on ORSM.

Fig. 2 Agarose gel electrophoresis of species-specific PCR of representative *R. dentocariosa* strains.

Lane M, molecular weight DNA ladder; lane 1, *R. aeria* JCM 11412; lane 2, *R. mucilaginoso* DY18; lane 3, *R. dentocariosa* AIu 17931; 4-11, isolated strains LX1, LX4, LX5, LX9, LX16, LX20, LX21, LX29, respectively; lanes a, m, d indicate the species-specific PCR results of *R. aeria*, *R. mucilaginoso* and *R. dentocariosa*, respectively.

Fig. 3 Phylogenetic tree based on the 16S rDNA sequences.

Phylogenetic tree showing the relationships between LX1, LX4, LX5, LX9, LX16, LX20, LX21, and LX29 and the reference strains. The bar, 0.02, represents the number of substitutions per nucleotide.

Fig. 4 Transposon insertion sites of each mutant.

Parentheses behind the strain names indicate the protein names encoded by the gene inserted by EZ-Tn5. Large white arrows indicate coding sequences and their lengths (bp). Transposon insertion sites are indicated by solid triangles. Small arrows indicate the primers used to confirm the EZ-Tn5 insertion sites (Table 1). Dashed lines with solid circles represent the start of the coding sequence. The dashed lines indicate the distance from the start of the coding sequence to the transposon insertion site.

Fig. 5 Agarose gel electrophoresis of the PCR to confirm transposon insertion sites. Lane M, molecular weight DNA ladder; lanes WT, wild type strain; lanes 1-7, transposon insertion mutants LF1 - LF7, respectively; primer sets 1-7 indicate the primer set numbers shown in Table 1.

Table 1 The primers used to confirm EZ-Tn5 insertion sites in each mutant

Primer set*	Primer name	Sequence
1	LF1KF	CCTCAAGCGCGAGCTCACCAG
	LF1KR	ACAGTTCGTCTGGACTGGAGCG
2	LF2KF	GATACACGGGGCGCAGCGTAG
	LF2KR	GCGCATCGTACCGCTTATTCTG
3	LF3KF	CCGATCGCCTACTATCAGAAG
	LF3K	GCTTTATCCGTCTGTTCAG
4	LF4KF	GATAGCGTAGCCCTGCCCCAG
	LF4KR	TCGGTGTACAGCAAGTGGGCG
5	LF5KF	GATGCACCGGTATCGATAGCG
	LF5KR	ATGTGTATTTCCGCCGGTCTG
6	LF6KF	TCCACGGCTCGGAACCAACCG
	LF6KR	TGGGCAACGCAGTAAGTCACG
7	LF7KF	GTTACCCCGCCTAAGCCTG
	LF7KR	CATCATAGAGGGCCGAATCAG

*A set of primers was designed to locate approximately 100 bp upstream and downstream of the transposon insertion position.

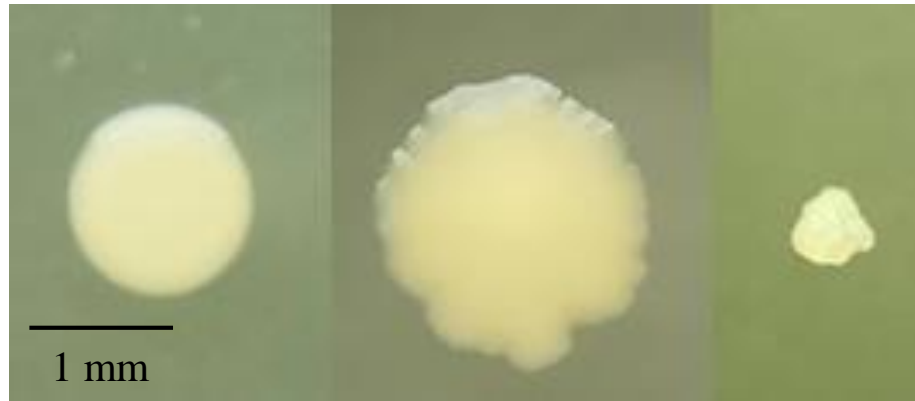
Table 2 Closest matched species estimated from 16S rRNA gene sequences

Strain	Closest match	Accession number	E-value
LX1	<i>Rothia dentocariosa</i>	MT512088.1	0
LX4	<i>Rothia dentocariosa</i>	CP054018.1	0
LX5	<i>Rothia dentocariosa</i>	CP054018.1	0
LX9	<i>Rothia dentocariosa</i>	MT512093.1	0
LX16	<i>Rothia dentocariosa</i>	CP054018.1	0
LX20	<i>Rothia dentocariosa</i>	MT512093.1	0
LX21	<i>Rothia dentocariosa</i>	MT512088.1	0
LX29	<i>Rothia dentocariosa</i>	MT512088.1	0

Table 3 Transposon insertion mutant stains obtained

5-FOA resistance clone	Taxon /annotation	Accession number	E-value
LF1	<i>Rothia dentocariosa</i> ATCC 17931/Antioxidant, AhpC/TSA family	ADP40600.1	8.00e-104
LF2	<i>Rothia dentocariosa</i> /TetR family transcriptional regulator	WP_314847624.1	1.00e-79
LF3	<i>Rothia dentocariosa</i> /LytR C-terminal domain-containing protein	WP_279752615.1	4.00e-80
LF4	<i>Rothia</i> sp. HMSC065G12/Pentapeptide repeat-containing protein	WP_070627589.1	6.00e-27
LF5	<i>Rothia</i> sp. HMSC065G12/Pentapeptide repeat-containing protein	WP_070627589.1	6.00e-27
LF6	<i>Rothia dentocariosa</i> /NAD(P)H-dependent oxidoreductase, partial	WP_315306179.1	2.00e-76
LF7	<i>Rothia</i> /MarR family transcriptional regulator	WP_004006328.1	1.00e-79

Fig. 1

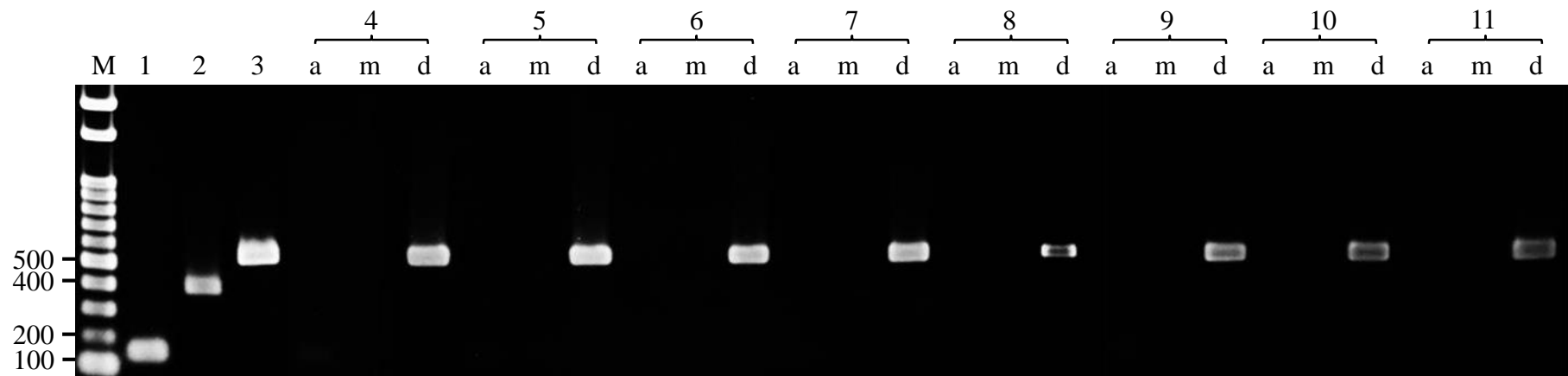


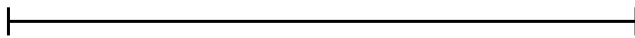
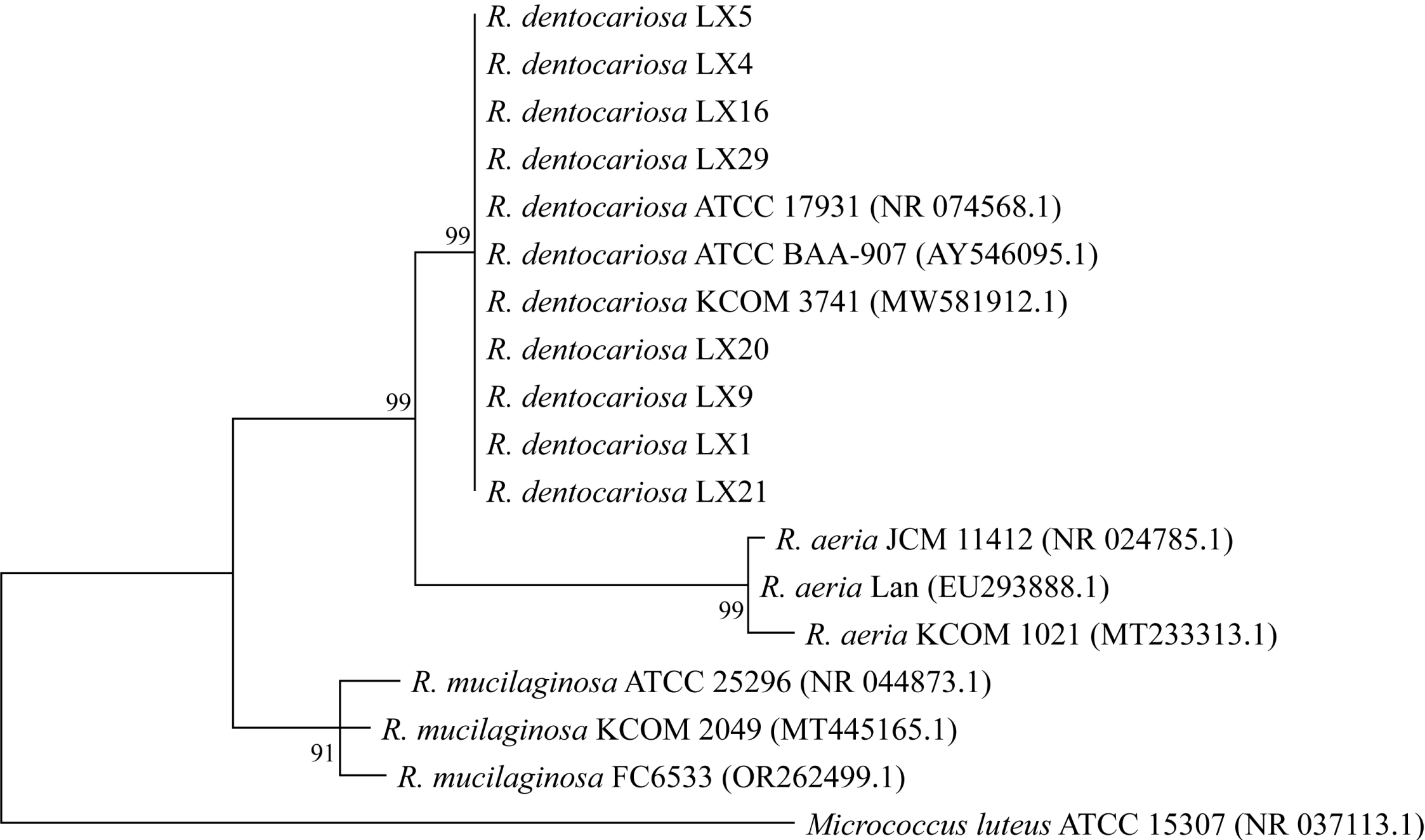
R. dentocariosa

R. mucilaginoso

R. aeri

Fig. 2





0.02

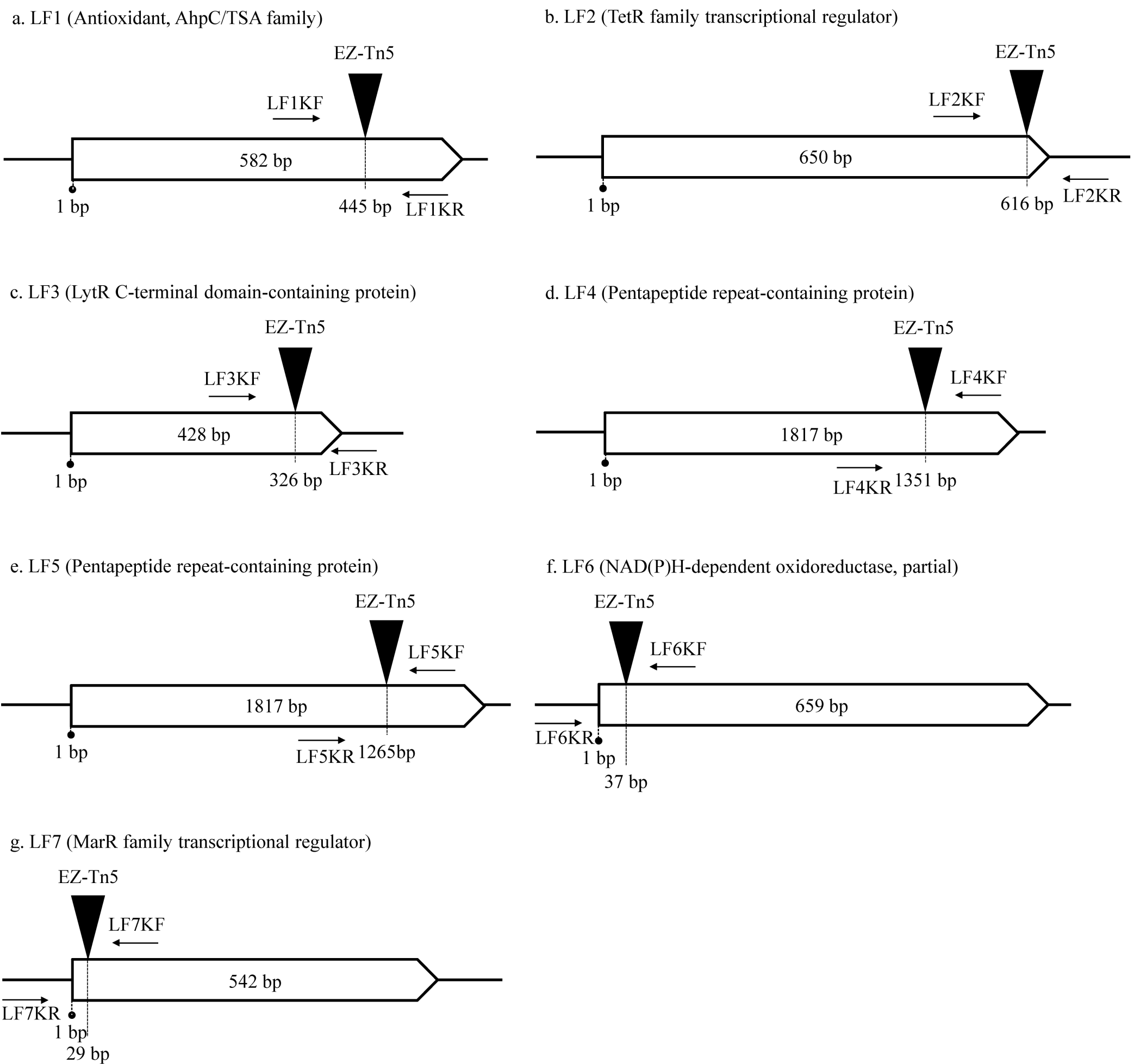


Fig. 5

