Journal of Oral Biosciences 68 (2026) 100718

Contents lists available at ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Evaluation of *Mycobacterium*-derived plasmids for application in oral *Actinomyces* species

Sakiko Ohara [a,b], Yijuan Sheng [a], Yuki Nishiya [a,c], Ikue Tosa [a,d], Katsuki Takebe [e], Yuki Arimura [c], Hiroshi Mese [b], Naoko Ohara [f], Naoya Ohara [a,d,g,*]

[a] Department of Oral Microbiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
[b] Department of Dentistry and Oral Surgery, Fukuyama City Hospital, Fukuyama, Japan
[c] Department of Oral and Maxillofacial Reconstructive Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
[d] Advanced Research Center for Oral and Craniofacial Sciences, Dental School, Okayama University, Okayama, Japan
[e] Department of Dental Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
[f] Department of Operative Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
[g] Research Center for Intestinal Health Science, Okayama University, Okayama, Japan

ARTICLE INFO

Keywords:
Actinomyces
Plasmid
Shuttle vector
Transformation

ABSTRACT

Objectives: Genetic manipulation tools are essential for elucidating the pathogenic mechanisms of microorganisms. Several species of *Actinomyces,* including *A. israelii,* are present in the oral cavity and they are the causative agents of actinomycosis. However, efficient gene-editing tools for these species have not yet been developed. In this study, the aim was to evaluate the introduction of foreign genes into *Actinomyces* using plasmids derived from *Mycobacterium*, which belong to the same class as *Actinomycetes*.
Methods: A truncated derivative of pYT923, pYT923S, which contains the replication origin of the *M. scrofulaceum* plasmid pMSC262 was constructed and introduced into *A. israelii* by electrotransformation.
Results: pYT923S was successfully introduced into *A. israelii*. The transformation efficiency of *A. israelii* was approximately 7–66 CFU/μg of DNA, and all transformed colonies harbored pYT923S. The plasmid recovered from *A. israelii* replicated in *Escherichia coli*.
Conclusions: pYT923S was introduced into and maintained within *A. israelii*. Therefore, the pYT923S vector represents a useful genetic tool for *Actinomyces* and it is expected to facilitate future studies on the biology and pathogenicity of *Actinomyces*.

1. Introduction

Actinomycetes are among the major constituents of the oral microbiota and are classified as Gram-positive, rod-shaped bacteria. *Actinomycetes* include *Streptomycetales*, *Mycobacteriales* and *Actinomycetales* [1]. *Actinomyces* species have been detected in the oral cavity of infants as early as 2 months after birth, suggesting that they contribute to the establishment of the oral microbiota at an early stage of life [2,3]. Within supragingival and subgingival biofilms, *Actinomyces* species participate in the early stages of microbial colonization [4,5].

In addition to their commensal role, *Actinomyces* species are major causative agents of cervicofacial actinomycosis in the head and neck region [6]. Actinomycosis is characterized by granulomatous and suppurative abscesses formation, subsequently progressing to multiple abscesses with sinus tract formation [6]. *Actinomyces israelii* is a representative pathogen that produces characteristic brown sulfur granules (also known as druses or bacterial colonies) [7]. Thus, *Actinomyces* species exhibit a broad biological spectrum in the oral cavity, contributing to normal microbiota formation and exhibiting pathogenic potential [8]. However, to our knowledge, no established virulence factor has been reported in *A. israelii*.

However, functional genomic analysis of genes in oral *Actinomyces* species remains largely unexplored, partly because of the lack of stable and efficient genetic tools for these organisms. Genetic engineering tools, such as the generation of gene deletion mutants and complementation strains, are essential to elucidate the pathogenicity and

* Corresponding author. Department of Oral Microbiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 2-5-1 Shikata-cho, Kita-ku, Okayama-city, Okayama, 700-8525, Japan.
E-mail address: oharan@md.okayama-u.ac.jp (N. Ohara).

https://doi.org/10.1016/j.job.2025.100718
Received 7 November 2025; Received in revised form 26 November 2025; Accepted 27 November 2025
Available online 2 December 2025

physiological characteristics of microorganisms. Although analyses using gene deletion mutants have been conducted in *Actinomyces*, there are very few reports on the construction of complementation strains [9–11].

In this study, we focused on pYT923, a shuttle vector constructed by inserting a fragment containing the replication region derived from pMSC262 of *Mycobacterium scrofulaceum*, a member of the same class *Actinomycetes,* into the *Escherichia coli* plasmid pACYC177 [12]. We generated a modified plasmid, pYT923S, capable of introducing foreign genes into *A. israelii* through genome editing of pYT923. Thus, pYT923S is a promising genetic tool for elucidating the pathogenic mechanisms and biological functions of *A. israelii*.

2. Materials and methods

2.1. Bacterial strains and growth conditions

E. coli DH5α was cultured aerobically in Luria-Bertani (LB) medium (Nacalai Tesque, Kyoto, Japan) at 37 °C. The *A. israelli* strain JCM12964 (RIKEN BioResource Research Center [BRC], Tsukuba, Japan) was cultured in BHIY consisting of 37 g/L Brain Heart Infusion (BHI) (BD, Franklin Lakes, NJ, USA), 0.5 g/L yeast extract (BD), and 0.5 % Tween 80 under anaerobic conditions. For *A. naeslundii* strain JCM8349 (RIKEN BRC), BHIY was used, except for Tween 80. Solid cultures were grown anaerobically on BHIY agar. Anaerobic conditions were maintained using AnaeroPack (Mitsubishi Gas Chemical Company, Tokyo, Japan) at 37 °C. When required, antibiotics were added at the following final concentrations: carbenicillin, 50 μg/mL; kanamycin (Km), 50 μg/mL.

2.2. DNA manipulation

Standard techniques were used for DNA manipulation unless otherwise indicated.

2.3. Plasmid construction

The pYT923 plasmid was kindly provided by Professor Hatsumi Taniguchi (University of Occupational and Environmental Health, Kitakyushu, Japan) [12]. pYT923S was constructed by digesting pYT923 with HincII and self-ligation to reduce the size of the plasmid.

2.4. Electrotransformation of Actinomyces cells with plasmid DNA

Actinomyces strains were anaerobically grown at 37 °C in BHIY medium overnight and kept on ice for 10 min. Cells were harvested, washed once with 20 mL of EP buffer consisting of 272 mM mannitol, 2 mM sodium phosphate buffer (pH 7.1), and 0.05 % Tween 80, and resuspended in the EP buffer to adjust the OD600 to 2.0. Aliquots (100 μL) of competent cells were mixed with 5 μg of plasmid DNA and incubated on ice for 30 min. The mixtures were then transferred into electroporation cuvettes (inter-electrode distance of 1 mm) (Nepa Gene, Chiba, Japan), which were electrically pulsed using a Gene Pulser II (BioRad, Hercules, CA, USA). Immediately after the electric pulse, 1 mL of BHIY was added to each sample and incubated anaerobically at 37 °C for 12 h. Cultures were spread onto BHIY agar plates containing Km. Electroporation of *Actinomyces* strains was performed once under the following conditions: 1700 V, 25 μF, and 600 Ω, with a bacterial cell number of 5.2×10^7 colony-forming unit (CFU). Transformation efficiency was calculated based on the number of colonies on the agar plates.

2.5. Polymerase chain reaction (PCR)

A. israelii colonies were suspended in 30 μL sterile water, and incubated for 15 min at 98 °C. After brief centrifuging, 5 μL were used for PCR. The PCR primers YT1 (5′-CGAGGGCCATGAACGCTTCAC-3′, positions 2,820 to 2,840) and YT2 (5′-AAGGTGGAGGAAGGTGATGTC-3′, positions 3,096 to 3,116), specific for pYT923S (Supplemental material 1), and PrimeSTAR GXL DNA polymerase (Takara Bio, Shiga, Japan) were used in this study. PCR consisted of 1 cycle of denaturation (98 °C, 20 s) followed by 30 cycles of amplification consisting of denaturation (98 °C, 10 s), annealing (58 °C, 15 s), and primer extension (68 °C, 1 min). The total volume of reaction mix was 20 μL. The predicted size of PCR product is 297 bp. After PCR reaction, 5 μL samples were loaded on 1 % agarose gel.

2.6. plasmid retention rate

To calculate the plasmid retention rate, 20 colonies from each plate were randomly selected and tested by PCR using YT1 and YT2 primers. The percentage of PCR-positive clones was defined as the plasmid retention rate.

2.7. DNA extraction from Actinomyces

A. israelii was suspended in 200 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Two hundred microliters of phenol/chloroform/isoamyl alcohol (25:24:1) was added to the suspension and gently mixed for 30 min, followed by centrifugation at 15,000 rpm for 15 min at 4 °C. The aqueous layer was transferred to a fresh tube, and one-tenth the volume of 3 M sodium acetate and 2.5 vol of ethanol were added. After incubation at 4 °C for 1 h, the solution was centrifuged at 15,000 rpm for 15 min at 4 °C. The DNA pellet was washed with 70 % ethanol and resuspended in 20 μL TE buffer.

3. Results

The plasmid pYT923S was successfully constructed by digesting pYT923 with HincII, containing the replication origin of pMSC262, the replication origin of *E. coli* plasmid p15A, and the Km resistance gene (Fig. 1A and B). Therefore, Km was used as the selection marker in subsequent experiments. The entire pYT923S sequence is shown in Supplemental material 1.

pYT193S plasmid was successfully introduced into *A. israelii* cells by electrotransformation, with a transformation efficiency of approximately 7–66 CFU/μg of DNA. Colonies that recovered from Km-containing plates (Fig. 2A) were analyzed using PCR with primers specific for pYT923S (Fig. 2B), and the plasmid retention rate was calculated. The average retention rate was 96.7 %, with a standard deviation of 2.9 %, indicating stable maintenance of pYT923S within *A. israelii*.

Furthermore, when plasmid DNA extracted from *A. israelii* was used to transform *E. coli*, colonies were obtained on LB agar plates containing Km. Plasmid DNA extracted from these *E. coli* colonies confirmed the presence of pYT923S **(**Supplemental material 2**)**, demonstrating that pYT923S can be introduced into *A. israelii* and replicate within this species. Similarly, pYT923S was successfully introduced into *A. naeslundii* **(**Supplemental material 3**)** with a transformation efficacy of approximately 20–30 CFU/μg DNA, and replication within this species was confirmed using the same *E. coli*-based method (data not shown). These results indicate that pYT923S can be introduced into at least two *Actinomyces* species and stably replicate within them.

4. Discussion

The transformation of *Actinomyces* species has long been considered technically challenging because circular plasmid DNA cannot be detected using the Anderson and McKay plasmid enrichment method [13]. Among the known vectors, pJRD215 is the only plasmid reported to enable the transformation of oral *Actinomyces,* such as *A. naeslundii* and *A. viscosus* by electroporation [10]. pJRD215 is a wide-host-range conjugative cosmid vector for Gram-negative bacteria that possesses a single replication origin [14].

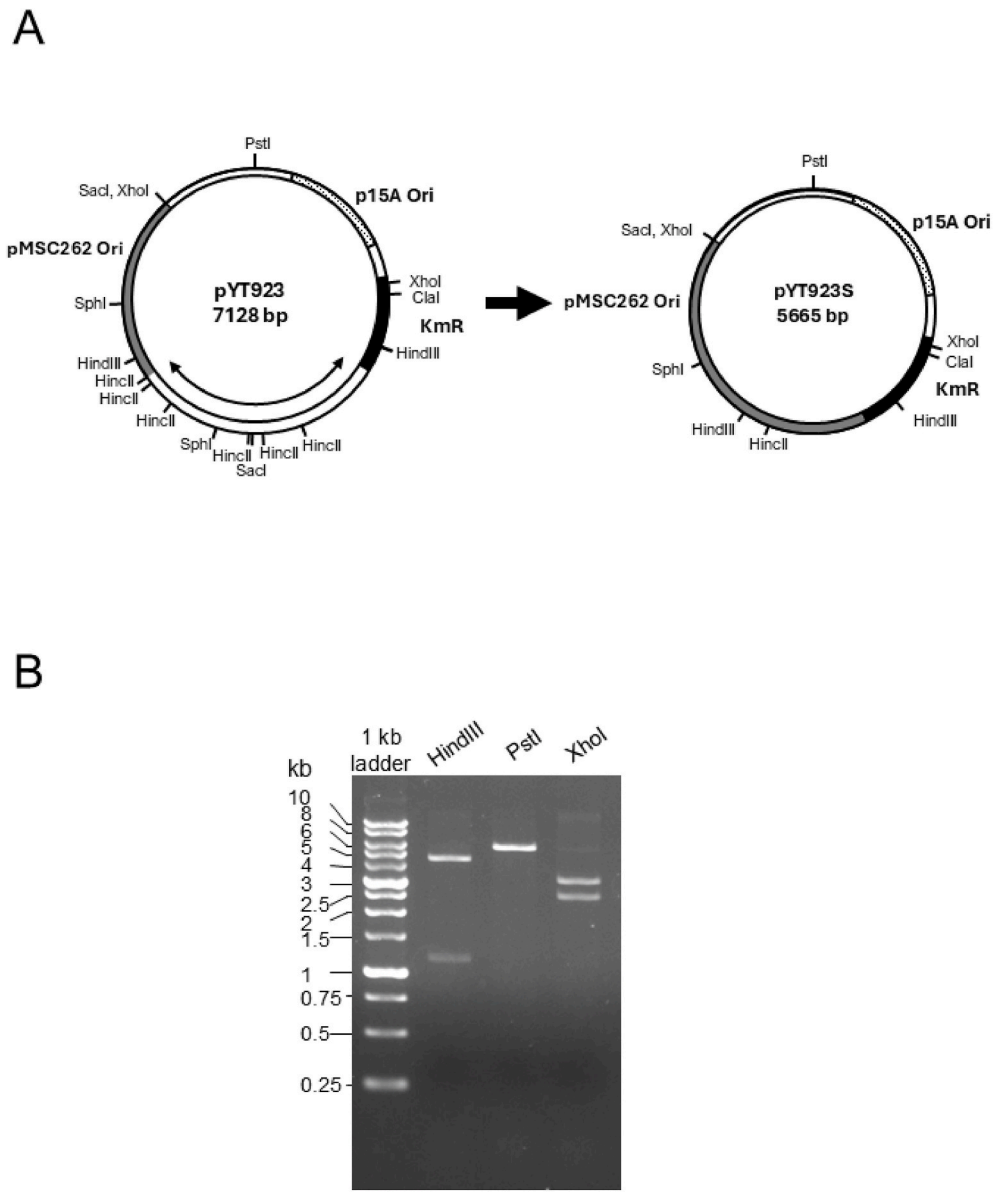


Fig. 1. A: Construction of shuttle plasmid pYT923S by digesting pYT923 using HincII. The double arrow indicates the region lost in pYT923S. KmR, kanamycin resistance gene; p15A Ori, replication origin of *E. coli* plasmid p15A; pMSC262 Ori, replication origin of *M. scrofulaceum* plasmid pMSC262. B: Restriction enzyme digestion pattern of pYT923S. Two hundred ng of pYT923S was digested with HindIII, PstI, and XhoI.

Both *Actinomyces* species previously transformed with pJRD215 are considered non-pathogenic oral commensals. In contrast, pYT923S was successfully introduced into *A. israelii*, an etiological agent of cervicofacial actinomycosis. Moreover, pYT923S has two origins of vegetative replication, one of which is derived from *Mycobacterium*, a member of the class *Actinomycetes*. These findings suggest that pYT923S is a promising candidate vector for the genetic manipulation of *Actinomyces* species. In BHIY medium, *A. naeslundii* did not aggregate, whereas *A. israelii* aggregated under the same conditions. The addition of 0.5 % Tween 80 enhanced the dispersion of *A. israelii* without causing significant cell lysis (data not shown). Consequently, all subsequent experiments, including cultivation and transformation of *A. israelii*, were performed in the presence of 0.5 % Tween 80.

However, unlike the pYT923S, which could not perform the conjugative transfer, in contrast to pJRD215, the transformation efficiency of *A. israelii* with pYT923S remained relatively low. Additionally, in *A. israelii*, the number of transformant colonies varied greatly among the plates. This variation was likely due to the incomplete dispersion of cell aggregates at the current concentration of Tween 80. Improving the dispersion of *A. israelii* cells and enhancing DNA uptake efficiency are important for establishing efficient transformation and vector systems in *A. israelii*.

The successful introduction of foreign DNA into *A. israelii* by electroporation not only demonstrates the feasibility of heterologous gene expression and the potential for genetic manipulation, including chromosomal gene disruption. This may facilitate the identification and functional analysis of pathogenic factors in this bacterium.

5. Conclusion

The shuttle vector plasmid pYT923S was successfully introduced into *A. israelii*. Therefore, the pYT923S vector represents a useful genetic tool for *Actinomyces* and is expected to facilitate future studies on *Actinomyces* biology and pathogenicity.

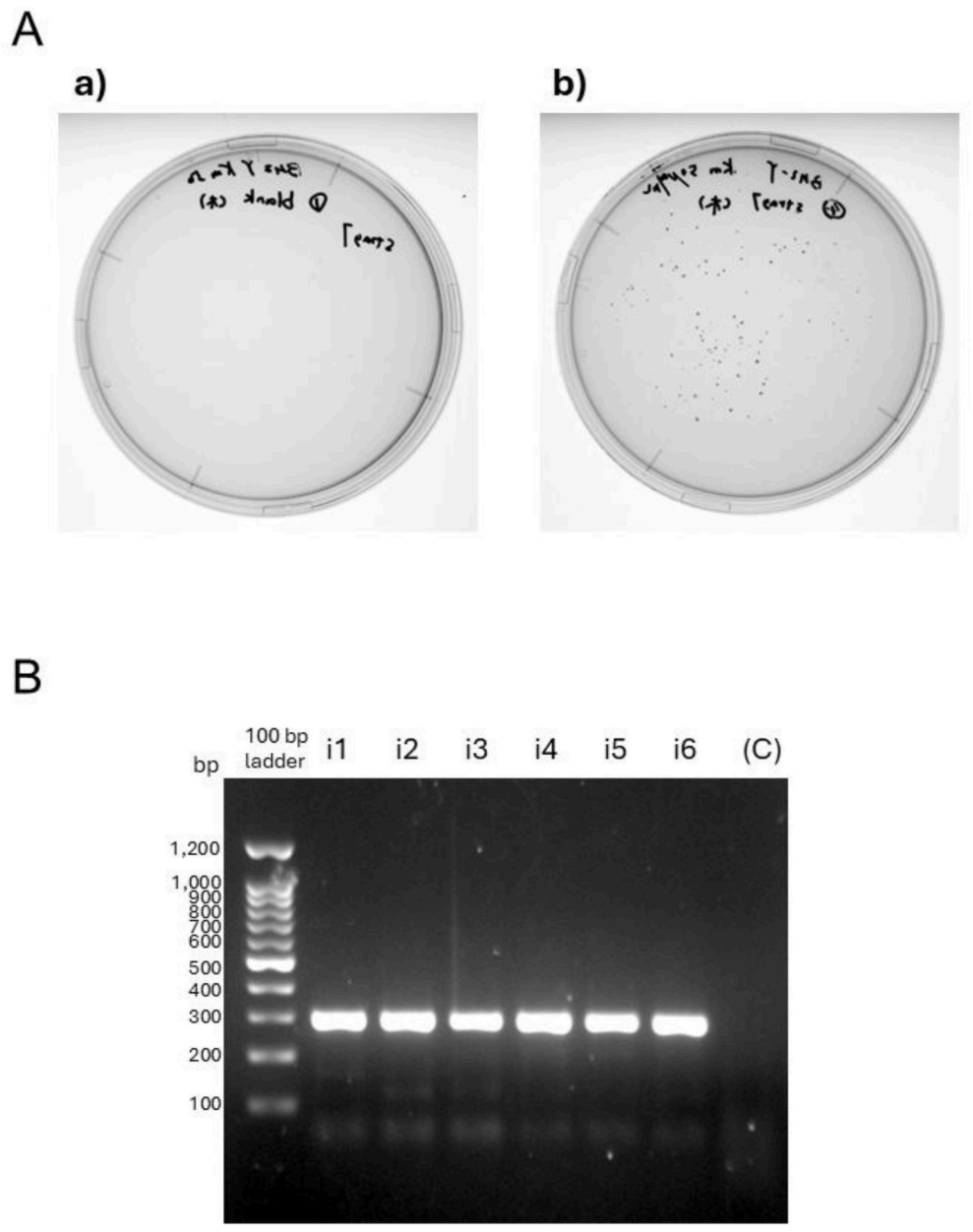


Fig. 2. A: Colonies of *A. israelii* JCM12964 transformed with pYT923S on BHIY plates containing 50 μg/mL kanamycin. a), without any plasmid; b), containing pYT923S. B: Detection of pYT923S in pYT923S-transformant using PCR. Six colonies were randomly selected from the pYT923S-transformants and subjected to PCR using primers specific to the pYT923S sequence. i1–i6 are pYT923S-transformants. (C) is *A. israelii* JCM12964.

Ethics approval and consent to participate

Not applicable.

Author contribution

Formal analysis: Sakiko Ohara, Yijuan Sheng, Naoya Ohara. Investigation: Sakiko Ohara, Yijuan Sheng, Yuki Nishiya, Ikue Tosa, Naoya Ohara. Methodology: Sakiko Ohara, Yijuan Sheng, Yuki Nishiya, Yuki Arimura, Naoko Ohara, Naoya Ohara. Validation: Sakiko Ohara, Yijuan Sheng, Yuki Nishiya, Ikue Tosa, Katsuki Takebe, Yuki Arimura, Hiroshi Mese, Naoko Ohara, Naoya Ohara. Writing – original draft: Sakiko Ohara, Katsuki Takebe, Naoya Ohara. Writing –review & editing: Sakiko Ohara, Yuki Nishiya, Ikue Tosa, Katsuki Takebe, Naoya Ohara. Conceptualization: Hiroshi Mese, Naoya Ohara.

Declaration of generative AI in scientific writing

No use generative AI and AI-assisted technologies in the writing process in the manuscript.

Declaration of competing interest

Authors have no conflict of interest to declare.

Acknowledgements

This study was partially supported by grants from the Japan Society for the Promotion of Science (JSPS, 23K0945905 to Naoko Ohara, 24K0261406 to Naoya Ohara). We would like to thank editage (Academic Proofing Service; https://www.editage.jp/) for the English language review.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.job.2025.100718.

References

[1] Nouioui I, Carro L, Garcia-Lopez M, Meier-Kolthoff JP, Woyke T, Kyrpides NC, et al. Genome-based taxonomic classification of the phylum *Actinobacteria*. Front Microbiol 2018;9:2007.

[2] Sarkonen N, Kononen E, Summanen P, Kanervo A, Takala A, Jousimies-Somer H. Oral colonization with *Actinomyces* species in infants by two years of age. J Dent Res 2000;79:864–7.

[3] Kononen E, Wade WG. *Actinomyces* and related organisms in human infections. Clin Microbiol Rev 2015;28:419–42.

[4] Zijnge V, van Leeuwen MB, Degener JE, Abbas F, Thurnheer T, Gmur R, et al. Oral biofilm architecture on natural teeth. PLoS One 2010;5:e9321.

[5] Kolenbrander PE, Palmer Jr RJ, Rickard AH, Jakubovics NS, Chalmers NI, Diaz PI. Bacterial interactions and successions during plaque development. Periodontol 2000. 2006;42:47–79.

[6] Valour F, Sénéchal A, Dupieux C, Karsenty J, Lustig S, Breton P, et al. Actinomycosis: etiology, clinical features, diagnosis, treatment, and management. Infect Drug Resist 2014;7:183–97.

[7] Pulverer G, Schütt-Gerowitt H, Schaal KP. Human cervicofacial Actinomycoses: microbiological data for 1997 cases. Clin Infect Dis 2003;37:490–7.

[8] Könönen E, Wade WG. *Actinomyces* and related organisms in human infections. Clin Microbiol Rev 2015;28:419–42.

[9] Wu C, Mishra A, Reardon ME, Huang IH, Counts SC, Das A, et al. Structural determinants of *actinomyces* sortase SrtC2 required for membrane localization and assembly of type 2 fimbriae for interbacterial coaggregation and oral biofilm formation. J Bacteriol 2012;194:2531–9.

[10] Yeung MK, Kozelsky CS. Transformation of *Actinomyces* spp. by a gram-negative broad-host-range plasmid. J Bacteriol 1994;176:4173–6.

[11] Mizuno H, Mashimo C, Fukushima H. Genetic analysis of a wide host-range conjugative plasmid pJRD215 and its application to *Actinomyces* spp. Shika Igaku (J Osaka Odontol Sci) 2011;74:66–77 (in Japanese).

[12] Goto Y, Taniguchi H, Udou T, Mizuguchi Y, Tokunaga T. Development of a new host vector system in mycobacteria. FEMS Microbiol Lett 1991;67:277–82.

[13] Anderson D, McKay LL. Simple and rapid method for isolating large plasmid DNA from lactic *Streptococci*. Appl Environ Microbiol 1983;46:549–52.

[14] Davison J, Heusterspreute M, Chevalier N, Ha-Thi V, Brunel F. Vectors with restriction site banks. V. pJRD215, a wide-host-range cosmid vector with multiple cloning sites. Gene 1987;51:275–80.

Supplementary material 1

Supplementary material 1. The entire nucleotide sequence of pYT923S (5,665 bp).

```
   1  CTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTT
  61  CCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCT
 121  CGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTC
 181  GCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACA
 241  CGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCT
 301  CACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATT
 361  TAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGA
 421  CCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCTTAATAAGATGAT
 481  CTTCTTGAGATCGTTTTGGTCTGCGCGTAATCTCTTGCTCTGAAAACGAAAAAACCGCCT
 541  TGCAGGGCGGTTTTTCGAAGGTTCTCTGAGCTACCAACTCTTTGAACCGAGGTAACTGGC
 601  TTGGAGGAGCGCAGTCACCAAAACTTGTCCTTTCAGTTTAGCCTTAACCGGCGCATGACT
 661  TCAAGACTAACTCCTCTAAATCAATTACCAGTGGCTGCTGCCAGTGGTGCTTTTGCATGT
 721  CTTTCCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGACTGAACG
 781  GGGGGTTCGTGCATACAGTCCAGCTTGGAGCGAACTGCCTACCCGGAACTGAGTGTCAGG
 841  CGTGGAATGAGACAAACGCGGCCATAACAGCGGAATGACACCGGTAAACCGAAAGGCAGG
 901  AACAGGAGAGCGCACGAGGGAGCCGCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGT
 961  CGGGTTTCGCCACCACTGATTTGAGCGTCAGATTTCGTGATGCTTGTCAGGGGGGCGGAG
1021  CCTATGGAAAAACGGCTTTGCCGCGGCCCTCTCACTTCCCTGTTAAGTATCTTCCTGGCA
1081  TCTTCCAGGAAATCTCCGCCCCGTTCGTAAGCCATTTCCGCTCGCCGCAGTCGAACGACC
1141  GAGCGTAGCGAGTCAGTGAGCGAGGAAGCGGAATATATCCTGTATCACATATTCTGCTGA
1201  CGCACCGGTGCAGCCTTTTTTCTCCTGCCACATGAAGCACTTCACTGACACCCTCATCAG
1261  TGCCAACATAGTAAGCCAGTATACACTCCGCTAGCGCTGAGGTCTGCCTCGTGAAGAAGG
1321  TGTTGCTGACTCATACCAGGCCTGAATCGCCCCATCATCCAGCCAGAAAGTGAGGGAGCC
1381  ACGGTTGATGAGAGCTTTGTTGTAGGTGGACCAGTTGGTGATTTTGAACTTTTGCTTTGC
1441  CACGGAACGGTCTGCGTTGTCGGGAAGATGCGTGATCTGATCCTTCAACTCAGCAAAAGT
1501  TCGATTTATTCAACAAAGCCACGTTGTGTCTCAAAATCTCTGATGTTACATTGCACAAGA
1561  TAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGGG
1621  TGTTATGAGCCATATTCAACGGGAAACGTCTTGCTCGAGGCCGCGATTAAATTCCAACAT
1681  GGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGAC
1741  AATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGG
1801  TAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTAT
1861  GCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCAC
```

1921 TGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAA
1981 TATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTG
2041 TCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGG
2101 TTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTG
2161 GAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTT
2221 CTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACG
2281 AGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTT
2341 TTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAA
2401 TAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGTT
2461 GTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGGCTTTGTTGAATAAATCGA
2521 ACTTTTGCTGAGTTGAAGGATCAGATCACGCATCTTCCCGACAACGCAGACCGTTCCGTG
2581 GCAAAGCAAAAGTTCAAAATCACCAACTGGTCCACCTACAACAAAGCTCTCATCAACCGT
2641 GGCTCCCTCACTTTCTGGCTGGATGATGGGGCGATTCAGGCCTGGTATGAGTCAGCAACA
2701 CCTTCTTCACGAGGCAGACCTCAGCGCTCAAAGATGCAGGGGTAAAAGCTAACCGCATCT
2761 TTACCGACAAGGCATCCGGCAGTTCAACAGATCGGGAAGGGCTGGATTTGCTGAGGATGA
2821 AGGTGGAGGAAGGTGATGTCATTCTGGTGAAGAAGCTCGACCGTCTTGGCCGCGACACCG
2881 CCGACATGATCCAACTGATAAAAGAGTTTGATGCTCAGGGTGTAGCGGTTCGGTTTATTG
2941 ACGACGGGATCAGTACCGACGGTGATATGGGGCAAATGGTGGTCACCATCCTGTCGGCTG
3001 TGGCACAGGCTGAACGCCGGAGGATCTGCGCCCGCACATCTTCGGCGTCCACAAGGTCCA
3061 ACACGGCCTGCTCTTCGTGGAGGAACACCCCGAGGGTGAAGCGTTCATGGCCCTCGCGTT
3121 GAGGCGCGGCCCGCGCCGCGGTGACCCGCATTCCCAGCGCGTCGACGAGTTGTCCGCTGA
3181 GCTGGCGCCGGCGGACCGGCGCGCCCTACGCGACGTCATCGCCAACAGCGTCTTAGAGGG
3241 CCACCACCCCACCGCCGGCGAGATCACCAATCTGGTCGCGTTCGCGGCCGGAAAGATCAG
3301 CATGGCCGACTACCTCACCCACGCCACCCACGCCGCCAAGCCGGGCGCTGCTAAGCGCAG
3361 CTAACAATCAAGCTTCGGGAAGAAGGTTAGGGCGGCTACCGAAGCGCAGTGGGCATCCGC
3421 CGGTGGCGCCATCCAGCGCACCCAGATACGTCACCGTGACCCAGGTTGGGCACCGCTTCG
3481 CGGGTCGATGATGGGAACATCACGCCAGCCCGCACGAAGCGGAGAACCGATGAGCGTCGA
3541 GGCGAAGGTGACCGAGCTGCTGGTGCGCCGCGCCCAGCCCGGTGGGGAACGGGTGCTCGA
3601 CTGGCTGGTGTACGGGACACCGCTGCCGCGGCTGCCGCGCCTCGACGTGATGTGCTGCGA
3661 TCAGCGCGACTGGCCGGGCCTCGGCGGCGACGGGCTCGACAACCACTAGCCCAGCGGCCC
3721 CGGCCCGCGCCGATGCGTGCGGGCCGCGGCCACCGCGGCCGCGCGCTGTGCGGCCTCTTC
3781 GGCGACGACGTGAGTGCGTTTGTGCGCGCCGCGGCGCGCCGCCTCGGCTGCGACCGCCCG
3841 CGCCGCGGCATGCCCAGGTCCGCTCAGCGCGGCGCGGCCCGCGGCGCGGGCGCGCTCATG
3901 CTCCCCGTGTGCGGCGCGCTGCGCGGCCAGGTGCGCGCGGTGTGCGGCACGCTCGGCGAC
3961 CTCGCGCGCCTCATCCAGGGCGGCCGGACGCTCGGCCAAGTGCTCGCGGCCATGCCAGGC
4021 CAGCATCGCGCCCAGCAGTCCGATCGGCTTGTGCGGGTGATCGGGGATGCGCCGGCCGGT

```
4081  CACCGCGGCCCAGTCGGTAATGAGCGCGTTGAGATCGCGCGGCGTCCAGCCATACGCGGC
4141  CGGCCCGGCCAACAGCGCCGCCCAAGCTCCAGCGGTGTGCCGGCGCGCCCACGGCGGGGC
4201  GTGCGCAGCGGCGCGCCAGGCCCGCGCCAGCGCCAACCCCCCGCCATCGGGTGCTCGGCG
4261  GCGCACAGCGCCGCCCTGCCGGCGGCCCGCAGGGCCACCGGCGCCGGTAGTAACCACGTC
4321  TTTACCGGAAGGTTGATCCCTTACGGGACCGCTTCGCGGGTGGGGTGACAGCCGCTGCAC
4381  AAACCGGGCCAGCTGCGGATTGTCGTGCAGCGCCCACACCGACGCCCAACCGCGACCGCG
4441  GTCCCCCACCCGCCAGGACGCCAGCCGCTCCACCCGGGTGCGCTGCCGGCCACGCAGCAC
4501  CTCAGTAGCCACCCCCAACAGCCGCAGCACAGTGTCAGCGCGCTGCACCGTACGCACCGA
4561  GTAGCCGGTATCGGCCGCCAGCCGCTCATTAGACGGCCGACAGTTCCGCCCGGTGGCGTG
4621  ATCGGCATAGCGGGCACGGGCCCGCGCGATCGCCAACAACGCTCGGCGGCTGATCGGGTT
4681  ACCCGGCATCACCGGACGCACCTGCGCGTCGTAGCGGCAGTCATAGGCCACCGCAACCGT
4741  CCACCTAGCCCACCGCTCAAGCCGCCCGGTCCAACACGGCACACCCGCATACGGGGCCTC
4801  GCCGAGCTCGAGCACAATCGCCGCGACAGCCGCCGCCTCGGATCGCTGGCGGTGCTCATG
4861  CTCGGCGGCCGCCAACCGCGCCGCCCACGGCCGCGTACACACCACACCACGACGCGGCCG
4921  CAGCGAAGTGCAGCCGCGATACGGCGGGCAAGCAATTTCGGAAAAGTCACGGTGTTGCGC
4981  GTCCTCTGGAACCTGAAACAGGTATGCGCTACCGTGAGACCTGCGATCCAAAGCAAGTCC
5041  CTTCGGGGAAGCGGAAACCCTCGGCGGCCAGGCCGGGGGTTTCGCCGTTTATTGAGCTGT
5101  CACATCTGCCCGGCGAAACGTTCAGCCAATGCGAGAGCCGGGCGGCTGCGGTCAGATCGC
5161  CAGCGGCAACACCTCCCTGTCGTTTAGCTCACGGACTAGATCAGGACGGCCCACATGCTG
5221  CGCAAGCACGTCGGCCGCGTACTGCGACATGCTCATGCCCAGCGCGGCCGCGCGTCGGCG
5281  GACTTCGTCATAGACGGGCAGTGTTGGGCGAACACCGATCAGGACCCGGTCGCCCTTGTG
5341  CGGTTGAGCCATAGAAAGATCCTTCCCCGCCGTAACTGTTATACGGCGACGACACGCCGT
5401  TAGGCCGTGATGTTTTCGGGATCAAGCTGTACAACGCTCTTGAAATACCAGGGCCGCAGA
5461  CGCCGGTGGCTTTCAGCAGCGCCGCGCCTTGCGCTCAGCTCGTCGGAGATTCTGGAGGCT
5521  AGCCGGCGAGATAATCGAGTATCTGCCGGCGTAGTTCATCGCTGCTCGATTCGGTAAGAG
5581  AGGCAAGCAGTTTCGGGCCATCGCCGTCACCGGTGGCGATGAGGCTAAGCAATAGGTGCT
5641  CGGTGCCGATGTGGTCGTGGTTGAG
```

Location of features

replication origin of *E. coli* plasmid p15A: 471…1,297

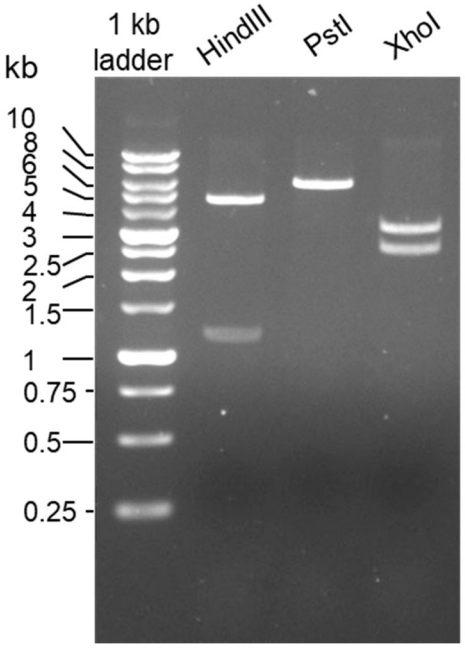
kanamycin resistance gene CDS: 1,625…2,440

replication origin of *M. scrofulaceum* plasmid pMSC262: 3,375 …5,655

primer YT1: 2,820…2,840

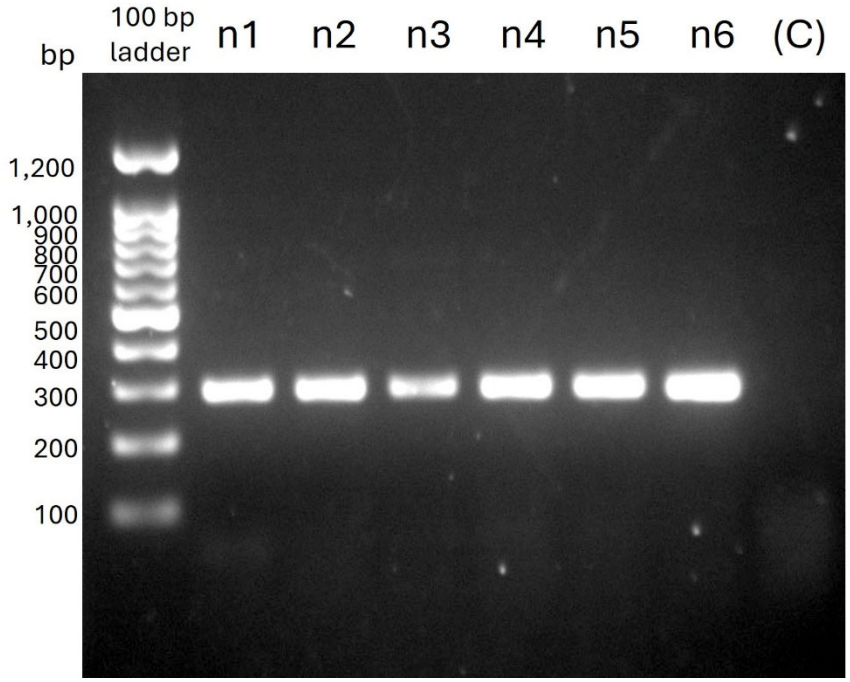
primer YT2: 3,096...3,116 (complement)

Supplemental material 2.



Supplemental material 2. Restriction enzyme digestion pattern of pYT923S obtained from pYT923-transformed *A. israelii*. DNA was extracted from pYT923-transformed *A. israelii* using the phenol extraction method described in Materials and Methods. The extracted DNA was then transformed into *E. coli* 5α, and the colonies that appeared on Km-containing LB agar were cultured in LB medium containing Km, and the plasmid was prepared. Two hundred ng of obtained plasmid was digested HindIII, PstI, and XhoI.

Supplemental material 3.



Supplemental material 3. Detection of pYT923S in pYT923S-transformed *A. naeslundii* using PCR. *A. naeslundii* JCM8349 was transformed with pYT923S and spread on BHIY plates containing 50 μg/mL kanamycin. Six colonies that appeared on the plates were randomly selected and subjected to PCR using primers specific to the pYT923S sequence. n1–n6 represent the pYT923S-transformants. (C) represents *A. naeslundii* JCM8349.