Characterization of the genes responsible for rubber degradation in Actinoplanes sp. strain OR16

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Abstract
A Gram-positive rubber-degrading bacterium, Actinoplanes sp. strain OR16 (strain NBRC 114529), is able to grow on agar plates containing natural and synthetic rubber as the sole sources of carbon and energy. When this strain was grown on natural rubber latex overlay agar plates, translucent halos around the cells were observed. To identify the natural rubber degradation genes and other features of its metabolism, its complete genome sequence was determined. The genome of OR16 consists of 9,293,892 bp and comprises one circular chromosome (GenBank accession number AP019371.1) with a G + C content of 70.3%. The genome contains 8238 protein-coding and 18 rRNA genes. A homology search of the genome sequence revealed that three genes (lcp1, lcp2, and lcp3) are homologous to an extracellular latex-clearing protein (Lcp) of Streptomyces sp. K30. RT-PCR analysis revealed that lcp1 and lcp2 seem to constitute an operon. Purified lcp gene products have oxygen consumption activity toward natural rubber latex, suggesting that all these genes encode rubber-degrading enzymes in OR16. Quantitative reverse transcription-PCR analysis indicated that the transcription of these genes is induced during the growth of OR16 on natural rubber. The genes located adjacent to lcp1 and lcp3, which code for a TetR/AcrR-type transcriptional regulator, can bind to the promoter regions of these lcp genes. It is suggested that the putative regulators play a role in regulating the transcription of the lcp genes. These results strongly suggested that three lcp genes are required for the utilization of natural rubber in strain OR16.

Key Points
• The complete genome sequence of Actinoplanes sp. strain OR16 was determined.
• Three lcp genes which are involved in the natural rubber degradation in OR16 were identified.
• Transcription of these lcp genes is induced during utilization of rubber in OR16.
• Two regulators, which bind to the promoter regions of lcp, were determined.

Keywords Actinoplanes · Complete genome sequence · Natural rubber degradation · TetR/AcrR-type transcriptional regulator

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Introduction
Natural rubber (NR) is a biopolymer containing poly(cis-1,4-isoprene) as a main component and is produced by over 2000 plants including Hevea brasiliensis (Backhaus 1985). NR is used globally in industry for tires, seismic isolation rubbers, medical gloves, and many other products. The waste of these products is permanently disposed and treated by combustion or landfill processes, which are hazardous to the environment. It is necessary to find an alternative treatment process for rubber waste. NR-degrading microorganisms, including Gram-positive and Gram-negative bacteria, have been identified and characterized in the past 20 years (Linos et al. 2000a; Linos et al. 2000b). Because of their rubber degradation ability, these bacteria are expected to be used as practical tools for the treatment process of rubber waste.
Microbial degradation of NR has been widely reported in Gram-positive bacteria such as *Streptomyces*, *Gordonia*, *Rhodococcus*, and *Nocardia* species and in only a few of Gram-negative bacteria including *Steroidobacter cummoxidans* 35Y and *Rhizobacter gumniphilus* NS21T (Bröker et al. 2008; Imai et al. 2011; Jendrossek and Reinhardt 2003; Sharma et al. 2018). Two major types of NR-degrading enzymes, rubber oxygenase (RoxA/RoxB) and latex-clearing protein (Lcp), have been characterized in Gram-negative and Gram-positive bacteria, respectively. Exception includes a Gram-negative bacterium, *Solimonas fluminis*, which has an lcp homologous gene (Birke and Jendrossek 2019). RoxA from strain 35Y is an *exo*-type diheme oxygenase that oxidizes poly(cis-1,4-isoprene) into the C15 tri-isoprenoid product 12-oxo-4,8-dimethyltrideca-4,8-diene-1-al (Braaz et al. 2004). On the other hand, RoxB degrades NR via *endo* cleavage of poly(cis-1,4-isoprene) (Birke et al. 2017). It has been reported that the two rubber oxygenases are involved in the NR degradation in Gram-negative bacteria including several myxobacteria (Birke et al. 2017). Recently, it has been reported that the functional RoxB and RoxA orthologs, LatA1 and LatA2, respectively, are essential for NR degradation by *R. gumniphilus* NS21T (Birke et al. 2018; Kasai et al. 2017).

The NR-degrading actinomycete, *Streptomyces* sp. strain K30 has an Lcp, which is a cofactor-independent oxygenase that catalyzes the oxidative *endo* cleavage of poly(cis-1,4-isoprene) into low-molecular-weight products containing aldehyde and keto end groups (Fig. 1) (Birke and Jendrossek 2014; Bröker et al. 2008). In contrast to Gram-negative rubber degraders, almost all rubber-degrading actinomycetes, including *Streptomyces*, *Nocardia*, and *Rhodococcus* species, except for *Gordonia polyisoprenivorans* VH2, have a single lcp homolog (Hiessl et al. 2012; Luo et al. 2014; Oetermann et al. 2018; Watcharakul et al. 2016).

Recently, a rubber-utilizing bacterium, strain OR16 (strain NBRC 114529), was isolated from a botanical garden in Japan (Imai et al. 2011). This strain is capable of growing on solid media containing NR or synthetic isoprene rubber (IR) and produces translucent halos on NR latex overlay agar plates. Taxonomic analysis revealed that strain OR16 belongs to the genus *Actinoplanes*. The genus *Actinoplanes* contains the rubber-degrading strains *A. missouriensis* NBRC 102363, *A. italicus* DSM 43146, and *A. utahensis* NBRC 13244 (Jendrossek et al. 1997); however, all the genes and their gene products responsible for rubber degradation have not yet been characterized genetically and biochemically. Here, the genome sequence analysis of strain OR16 identified three lcp homologous genes which are responsible for the rubber utilization of this strain. Multiple lcp genes were characterized in *G. polyisoprenivorans* VH2, but the two lcp genes are located in chromosome and plasmid. On the other hand, three lcp genes of strain OR16 were located on the chromosome. Recently, three lcp homologous genes have been identified in *Streptomyces* sp. strain CFMR7 by the genome sequence analysis (Nanthini et al. 2017). However, the functions of the genes have not been characterized at the molecular level, and the roles of their gene products are not clear. To gain insight into the rubber degradation system including three lcp homologous genes and the characterization of the functions of multiple lcp genes of *Actinoplanes* sp. strain OR16 is important. This is the first report of the functional characterization of rubber-utilizing bacterium which has three lcp genes.

### Materials and methods

#### Bacterial strains, plasmids, and culture conditions

*Actinoplanes* sp. strain OR16 and its mutant derivatives were routinely grown at 30 °C in PYM medium (0.5% *Bactopeptone*, 0.3% *yeast extract*, and 0.1% MgSO₄·7H₂O; pH 7.0) or W minimal salt medium (Araki et al. 2011) containing 10 mM sodium succinate. Growth on NR or IR was examined using rubber overlay agar plates. To prepare the rubber overlay agar plates, W agar medium containing deproteinized NR (Chaikumpollert et al. 2012) or synthetic isoprene rubber at a final concentration of 0.4% (v/v) was overlaid to form a thin layer on a solid medium. *Escherichia coli* strains were cultivated aerobically at 30 °C or 37 °C in LB medium. If necessary, ampicillin (100 mg/l), kanamycin (25 mg/l), chloramphenicol (25 mg/l), tetracycline (10 mg/l), and apramycin (100 mg/l) were added to the medium.
DNA manipulations, nucleotide sequencing, and sequence analysis

DNA manipulations including total DNA isolation and nucleotide sequencing were performed as previously described (Masai et al. 1995). Analysis of nucleotide sequences was carried out as previously described (Kasai et al. 2009). The genome sequence of OR16 was determined by the combination of MiSeq, PacBio RS II system, and Sanger sequencing. These sequencing data were assembled by CLC genomics workbench (Qiagen). Annotation was performed using NCBI Prokaryotic Genome Annotation Pipeline ver.3.1 (Tatusova et al. 2016) and RAST server (Aziz et al. 2008). Genome sequences of strain OR16 and of related Actinoplanes spp. were compared by calculating average nucleotide identity (ANI) values using the JSpecies Web Service (Richter et al. 2016). Tat signal sequences were predicted using the TatP 1.0 software (http://www.cbs.dtu.dk/services/TatP/) (Bendtsen et al. 2005).

Expression of lcp genes in E. coli

The coding regions of lcp1, lcp2, and lcp3 were independently amplified by PCR using lcp1_F and R, lcp3_F and R, and lcp2_F and R primer pairs (Table 1). Each forward primer contained an NdeI site at the start codon of the corresponding target gene. The amplified fragments were separately cloned in pJET1.2 to generate the NdeI fragment containing the entire target gene. The NdeI fragments containing either of lcp genes were individually cloned in the expression vector, pET23b. The resultant plasmids were independently introduced into E. coli Rosetta-gami B(DE3)pLysS, and the transformants were grown at 30 °C in LB medium containing kanamycin, chloramphenicol, and tetracycline. When the absorbance at 600 nm (A600) of the culture reached 0.5, 1 mM isopropyl-β-D-thiogalactopyranoside was added, and the cultures were further incubated at 20 °C for 16 h. The resulting cells were harvested and resuspended in 50 mM Tris-HCl (pH 7.4). The cell extract was prepared by using French pressure cell press. After centrifugation, the supernatant was applied to a Ni Sepharose 6 Fast Flow column (GE Healthcare, Buckinghamshire, UK) previously equilibrated with buffer A consisting of 50 mM Tris-HCl (pH 7.5), 500 mM NaCl, and 100 mM imidazole. Proteins were allowed to bind for 1 min at 4 °C while rotating, followed by washing five times in 5 ml of buffer A. Histagged proteins were eluted with 5 ml of buffer B consisting of 50 mM Tris-HCl (pH 7.5), 500 mM NaCl, and 500 mM imidazole, and the fractions were pooled and concentrated.

Enzyme assays

The activities of Lcp1, Lcp2, and Lcp3 were assayed by measuring the substrate-dependent oxygen consumption rate. Each 2-ml assay mixture contained 50 mM Tris-HCl buffer, NR (final concentration was 0.2%), and purified enzyme (25 μg of protein). The reaction mixture was incubated at 30 °C, and the oxygen consumption rate was determined with an oxygen electrode (Dual Digital Model 20; Rank Brothers Ltd., Cambridge, England). One unit of enzyme activity was defined as the amount of activity that resulted in consumption of 1 μmol of O2 per 1 min. Specific activity was expressed in units per milligram of protein. The optimal pH and optimal temperature for Lcp1, Lcp2, and Lcp3 were determined at pH and temperature ranges of 6.0 to 8.5 and 25 to 40 °C, respectively, by using 50 mM Tris-HCl buffer.

| Table 1 | Primer sequences used in this study |
|---------|------------------------------------|
| Oligo nucleotide | Sequence (5’ to 3’) |
| lcp1_F | CATATGGAAACCTGACAGGCG |
| lcp1_R | CTCAAGGCTCGCGCAGGTG |
| lcp2_F | CATATGAACCCAGAAGGCTC |
| lcp2_R | AAGCTTTTCGGCGCTTTAG |
| lcp3_F | CATATGCAAATCTCAGCAGG |
| lcp3_R | GACCGGCCTGAAAGCTC |
| lcp1_lcp2_F2 | GGCTGTCGCGGATGTC |
| lcp1_lcp2_R2 | CACCTGCTGTCAGGAAAG |
| lcp2_oxiB_F2 | GATCATGCCAGAAGGACAT |
| lcp2_oxiB_R2 | GTTTGAGATGAGTCCGCA |
| oxiB_oxiA_F2 | CGTCAAGCTCGTCGAGAAG |
| oxiB_oxiA_R2 | TGGATGACGTGAGAAGC |
| QRTlcp1_F | GACGTCACTTTCAACAGAG |
| QRTlcp1_R | TAGAGAACCGCGAGTAG |
| QRTlcp1_Probe | CCGTCGACTTTCAACAGAA |
| QRTlcp2_F | GACGTCACTTTCAACAGAG |
| QRTlcp2_R | TAGAGAACCGCGAGTAG |
| QRTlcp2_Probe | CCGTCGACTTTCAACAGAA |
| QRTlcp3_F | CGTCAAGCTCGTCGAGAAG |
| QRTlcp3_R | TGGATGACGTGAGAAGC |
| QRTlcp3_Probe | CCGTCGACTTTCAACAGAA |
| QRT16sOR16_F | AGCGTTGTCCCGGATTTT |
| QRT16sOR16_R | CACCACTGCTGTCGAGAAG |
| QRT16sOR16_Probe | GACGTCACTTTCAACAGAA |
| tetR_lcp1_F | GGCGCCGAGTAACCCATC |
| tetR_lcp1_R | CGATGTAAGGAGACCGTC |
| lcp3_UP_F | CAGCGGCAGCCGAGCATG |
| lcp3_UP_R | CATTGGCTTACCTACTTAC |
Quantitative reverse transcription-PCR (qRT-PCR) analysis

The cells of OR16 were grown on a NR-overlay agar medium or W agar medium containing 10 mM sodium succinate at 30 °C for 3 days. They were collected by centrifugation and washed with 0.9% NaCl. The total RNA was extracted from the cells with ISOGEN II (Nippon Gene Co., Ltd., Tokyo, Japan) according to the manufacturer’s instructions and was treated with RNase-free DNase I (Roche). Single-stranded cDNA was synthesized from 1 μg of total RNA with 100 U of PrimeScript II reverse transcriptase (Takara Bio Inc., Otsu, Japan) and random hexamer primers in a 30 μl reaction mixture. The cDNA was subjected to RT-PCR and qRT-PCR analyses. These PCR analyses were carried out using the specific primers (Table 1) according to the previous reports (Kasai et al. 2017).

Electrophoretic mobility shift assays (EMSAs)

DNA fragments containing the upstream region from lcp1 and lcp3 were prepared by PCR with tetR_lcp1_F/R and lcp3_UP_F/R primer pairs, respectively (Table 1). The 3’ ends of the probe fragments were labeled with DIG-11-μlcp3_UP_F/R primer pairs, respectively (Table 1). The 3’ ends of the probe fragments were labeled with DIG-11-lcp3_UP_F/R primer pairs, respectively (Table 1). The 3’ ends of the probe fragments were labeled with DIG-11-lcp3_UP_F/R primer pairs, respectively (Table 1). The 3’ ends of the probe fragments were labeled with DIG-11-lcp3_UP_F/R primer pairs, respectively (Table 1). The 3’ ends of the probe fragments were labeled with DIG-11-lcp3_UP_F/R primer pairs, respectively (Table 1). The 3’ ends of the probe fragments were labeled with DIG-11-lcp3_UP_F/R primer pairs, respectively (Table 1).

Identification of the lcp orthologs in strain OR16

To identify the rubber-degrading genes in strain OR16, the genome sequence of strain OR16 (AAAR5849) of Streptomyces sp. strain K30 as the query, and three homologous genes, ACTI_59630, ACTI_59640, and ACTI_69520, were identified as the most closely related gene sequences; these genes were designated lcp1, lcp2, and lcp3, respectively (Fig. 2a). These results identified the amino acid sequences of the lcp1, lcp2, and lcp3 gene products shared overall identities of 56.6%, 53.9%, and 47.6% with that of Lcp from strain K30, respectively. The amino acid sequence identities among biochemically characterized Lcps from actinomycetes are summarized in Table 2. The deduced amino acid sequences encoded by ACTI_59650 and ACTI_59660 exhibited 71.6% and 78.0% identity with those of oxib and oxia, respectively; oxib and oxia are involved in degradation of low-molecular-weight rubber-degrading compounds in Streptomyces sp. strain K30 (Rose et al. 2005). In contrast, no other known genes, which may be involved in rubber degradation, were detected in the vicinity of the lcp3 gene. ACTI_59620 and ACTI_69510, encoding putative TetR/AcrR-type transcriptional regulators (TATRs), were located adjacent to lcp1 and lcp3, respectively (Fig. 2a). Hence, there is a possibility that TATRs seem to be involved in the transcriptional regulation of the rubber-degrading genes.

To define the operon structure of the lcp genes, RT-PCR analysis was performed with total RNA extracted from strain OR16 grown with NR latex. RT-PCR amplification products of the expected size were detected in the intergenic regions of lcp1-lcp2, lcp2-oxib, and oxib-oxia (Fig. 2b). These results suggest that the lcp1, lcp2, oxib, and oxia genes are organized in the same transcriptional unit.
**Heterologous expression of the lcp genes in E. coli**

To examine the roles of Lcp1, Lcp2, and Lcp3, each gene was fused with a 10× histidine-tag and introduced into E. coli Rosetta-gami B(DE3)pLysS cells. When the cell extracts from E. coli expressing each gene were prepared and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), the presence of proteins of corresponding sizes was observed (Fig. 3a–c). To characterize the activity toward NR latex, each of these his-tagged proteins (Lcp1-his, Lcp2-his, and Lcp3-his) was purified by Ni-affinity column chromatography. When the purified proteins were incubated with NR latex, oxygen consumption activity was observed. No consumption of oxygen was observed without protein or NR latex, strongly suggesting that these enzymes are involved in rubber degradation. The optimal temperature and optimal pH for the oxygen consumption activity of Lcp1-his, Lcp2-his, and Lcp3-his with NR latex were determined to be 30 °C and 7.0, 35 °C and

### Table 2

| Lcp | Lcp1OR16 | Lcp2OR16 | Lcp3OR16 | LcpK30 | Lcp1VH2 | Lcp2VH2 | LcpSH22a | LcpRPK1 |
|-----|----------|----------|----------|--------|---------|---------|----------|---------|
| Lcp1OR16 | 100% | 52.1% (213/409) | 49.4% (205/415) | 56.6% (231/408) | 64.4% (266/413) | 57.0% (235/412) | 59.5% (244/410) | 72.4% (296/409) |
| Lcp2OR16 | 100% | 53.9% (221/410) | 53.9% (221/410) | 59.6% (231/408) | 53.9% (221/410) | 53.9% (221/410) | 59.6% (231/408) | 53.9% (221/410) |
| Lcp3OR16 | 100% | 47.6% (196/412) | 47.6% (196/412) | 53.9% (221/410) | 47.6% (196/412) | 47.6% (196/412) | 53.9% (221/410) | 47.6% (196/412) |

* LcpK30, Lcp from Streptomyces sp. K30 (AAR25849); Lcp1VH2 and Lcp1VH2, Lcps from G. polyisoprenivorans VH2 (ABV68923 and AFA76036); LcpSH22a, Lcp from Nocardia nova SH22a (WP025350295); LcpRPK1, Lcp from R. rhodochrous RPK1 (AMY60409)
The specific activities of Lcp1-his, Lcp2-his, and Lcp3-his were 4.02 ± 0.65 (30 °C, pH 7.0), 1.17 ± 0.07 (35 °C, pH 7.5), and 0.22 ± 0.01 (30 °C, pH 6.5) U/mg protein, respectively (see Fig. S1 in the supplemental material).

Transcriptional induction of the lcp genes

To determine whether transcription of the lcp genes is induced in response to NR, the mRNA levels of lcp1, lcp2, and lcp3 were measured by quantitative RT-PCR (qRT-PCR) analysis using the total RNA harvested from cells grown on succinate with or without NR latex. Cells grown on succinate exhibited extremely low transcription levels of these three genes. In contrast, the transcription levels of lcp1, lcp2, and lcp3 in cells grown with NR latex were 22.2-fold, 17.1-fold, and 335-fold higher, respectively, than those in cells grown without NR latex (P < 0.05 by Student’s t test) (Fig. 4). These results strongly suggested that the transcription of these lcp genes is induced during NR utilization in OR16.

Binding ability of the regulators of the lcp genes

To confirm the role of the ACTI_59620 and ACTI_69510 gene products in regulating the transcription of the lcp genes, these genes were expressed in E. coli BL21(DE3) cells as histidine-tagged proteins (ACTI_59620-his and ACTI_69510-his). To characterize the DNA binding ability of ACTI_59620-his and ACTI_69510-his, these proteins were purified to near homogeneity (Fig. 3d and e). Purified ACTI_59620-his and ACTI_69510-his were used in electrophoretic mobility shift assays (EMSAs) with 199-bp and 203-bp DNA probes containing the upstream regions from lcp1 or lcp3, respectively (Fig. 5). As shown in Fig. 5a, ACTI_59620-his was able to bind to the upstream region from lcp1. Furthermore, a weakly shifted band was observed when the upstream region from lcp3 was used as probe (Fig. 5b). The
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The regulation of lcp genes seemed to be regulated by ACTI_59620 and ACTI_69510. The results suggested that ACTI_59620 mainly bound to the promoter of lcp1 to regulate transcription. In contrast, ACTI_69510-his and DNA complexes were formed when the regions upstream from lcp1 and lcp3 were used as probes (Fig. 5d and e). This indicated that ACTI_69510 is able to interact with both promoters of lcp1 and lcp3. Based on these results, the transcriptional induction of the lcp1 operon seemed to be regulated by ACTI_59620 and ACTI_69510. The regulation of lcp3 transcription mainly requires the binding of ACTI_69510 to the promoter.

Discussion

Multiple lcp homologs have rarely been identified in rubber degraders such as G. polyisoprenivorans VH2. The lcp1 and lcp2 genes are located on the chromosome and on the plasmid of VH2, respectively (Hiessl et al. 2012). On the other hand, lcp1, lcp2, and lcp3 are all located on the chromosome in OR16. In particular, the lcp1 and lcp2 genes are tandemly located in the same orientation (Fig. 2a). The organization of the lcp genes is similar to that of the lcp genes in Streptomyces sp. strain CFMR7 (Nanthini et al. 2017). The deduced amino acid sequences of the lcp1, lcp2, and lcp3 gene products of strain OR16 have 63.3%, 60.0%, and 59.2% identity, respectively, with those of the corresponding lcp genes from strain CFMR7. Hence, the lcp genes of strain CFMR7 might be involved in the rubber utilization as well as those of strain OR16. Because Lcp was predicted to be secreted via the twin arginine translocation (Tat) pathway (Hiessl et al. 2012; Yikmis et al. 2008), it is critical to identify the Tat signal sequences in Lcp1, Lcp2, and Lcp3. In OR16, the RRxxLx motifs of Lcp1, Lcp2, and Lcp3 were identified between the 6th and 10th, 3rd and 7th, and 6th and 10th residues, respectively. The putative cleavage sites of the Tat signal sequences, which contain the AxA motifs of Lcp1, Lcp2, and Lcp3, were located between 30th and 31st, 26th and 27th, and 33rd to 34th residues, respectively. Therefore, the lcp gene products might be secreted via the Tat pathway in strain OR16.

When NR was incubated with purified Lcps, the oxygen consumption activity was observed. The enzymatic activities are comparable with those of other reported Lcps in strain K30 (4.6 U/mg), strain VH2 (1.3 U/mg), and R. rhodochrous RPK1 (3.1 U/mg) (Birke et al. 2015; Hiessl et al. 2014; Watcharakul et al. 2016). Therefore, these Lcps might be involved in the rubber degradation by OR16 (Fig. 1). The activity of Lcp1 was approximately 7 times and 18 times higher than those of Lcp2 and Lcp3, respectively, suggesting that Lcp1 is mainly involved in rubber degradation in strain OR16. Unlike the enzymatic activities of the Lcp enzymes, the transcription level of lcp3 is significantly higher than those of lcp1 and lcp2 (P<0.005 by Student’s t test). According to their transcription levels, the role of lcp3 is thought to also be important for the rubber degradation.

RT-PCR analysis revealed that oxiB and oxiA homologous genes (ACTI_59650, ACTI_59660) tandemly located in the downstream region from lcp2 were cotranscribed with the lcp1 and lcp2 genes during rubber utilization. It has been reported that rubber degradation intermediate, low-molecular-weight aldehyde compounds are further metabolized by oxiAB, encoding oxidoreductase, in Streptomyces sp. strain K30 (Rose et al. 2005). It was suggested that ACTI_59650 and ACTI_59660 are involved in the catabolism of aldehyde intermediates for the utilization of rubber by strain OR16.

The ACTI_59620 gene encodes a putative TATR and is located upstream from lcp1 in the opposite direction (Fig. 2a). The deduced amino acid sequence of this gene product is similar to those of TATRs from Rhodococcus sp. Q15 (AAK97457) and R. opacus B-4 (BAH50508). It has been reported that TATRs regulate the transcription of genes involved in antibiotic resistance, antibiotic biosynthesis, catabolic pathways, and biofilm formation in Gram-negative and Gram-positive bacteria as a repressor or an activator (Pompeani et al. 2008; Ramos et al. 2005). Based on these facts, ACTI_59620 seemed to be involved in the regulation of lcp transcription. Furthermore, ACTI_69510, located adjacent to lcp3 and encoding a putative TATR, was found (Fig. 2a). ACTI_69510 seemed to be involved in lcp3 transcription. Interestingly, the amino acid sequences of the N-terminal (residues 22 to 196) and C-terminal (residues 201 to 401) regions of ACTI_69510 exhibited 25.0% identity with each other.
addition, a TATR superfamily-specific domain was found in each region, suggesting that ACTI_69510 was formed by duplication of the regions.

LcpRBA3(2) and LcpRVH2 were identified as transcriptional regulators for putative lcp gene expression in S. coelicolor A3(2) and G. polyisoprenivorans VH2, respectively (Coenen et al. 2019; Oetermann et al. 2019). These promoter regions of lcp1VH2 and lcp2VH2, which interacted with LcpRVH2, contained palindromic sequences, 5′-GATGTTACAACGTTACTCGGTTGTTACATC-3′ and 5′-GATACAGAGAAGCATAGACTGTAACTCGGTTTC-3′, respectively. In the binding region of ACTI_59620, a palindromic sequence (5′-GATGCGAATTTGTAACAGCGTATCAGCAATC-3′), which is similar to the sequence found in the lcp1VH2 promoter region, was identified. Due to the amino acid sequence similarity (48.7% identity) between LcpRVH2 and ACTI_59620, the formation of a protein-DNA complex by ACTI_59620 requires the palindromic sequence, similar to LcpRVH2.

The amino acid sequence of ACTI_69510 shared 72.3% identity with that of LcpRB A3(2) which binds to the promoter region of lcpA3(2) in strain A3(2) (Coenen et al. 2019). It has been reported that the LcpRB A3(2)-binding sequence contains a palindromic sequence (5′-TATGTTAATGAAAAATCACA-3′). In the upstream region from lcp3, a similar sequence (5′-TATGTTAATGAAAAATCACA-3′) was found. Therefore, the palindromic sequence seemed to be involved in the binding of ACTI_69510. Surprisingly, ACTI_69510 bound not only to the lcp3 promoter but also to the lcp1 promoter. However, a palindromic sequence similar to the palindromic sequence in the lcp3 promoter was not detected in the upstream region from lcp1. Because several palindromic sequences were identified in the lcp1 promoter region, the other sequence seemed to be involved in ACTI_69510 binding. Because ACTI_69510 has two helix-turn-helix DNA-binding motifs that are similar to each other (31.9% identity), ACTI_69510 might be able to recognize several palindromic sequences, including the lcp1 and lcp3 promoters, for its binding. The broad binding specificity of ACTI_69510 is thought to be effective in inducing the transcription of the lcp genes and reasonable for the efficient utilization of rubber in strain OR16.

In this study, we identified the rubber-degrading genes of strain OR16. This strain possesses three lcp genes which might be required for the utilization of natural rubber. Furthermore, two TATRs that have the ability to bind to the
promoter regions of the lcp genes were identified. Further analysis including deletion of the TATR-coding genes and the lcp promoter assay will enable us to clarify the actual function and contribution of these TATRs to the transcriptional regulation of rubber-degrading genes.

Author's contributions DK, AS, and MF conceived the project. NG and DK wrote the manuscript. TA, SK, and DVL generated all the physiologic data. NG sequenced the genome and assembled the genome presented here. NG and TA annotated and analyzed the genome of this strain. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

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