

Insights into the Microbial Degradation of Rubber and Gutta-Percha by Analysis of the Complete Genome of *Nocardia nova* SH22a

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The complete genome sequence of *Nocardia nova* SH22a was determined in light of the remarkable ability of rubber and gutta-percha (GP) degradation of this strain. The genome consists of a circular chromosome of 8,348,532 bp with a G+C content of 67.77% and 7,583 predicted protein-encoding genes. Functions were assigned to 72.45% of the coding sequences. Among them, a large number of genes probably involved in the metabolism of xenobiotics and hardly degradable compounds, as well as genes that participate in the synthesis of polyketide- and/or nonribosomal peptide-type secondary metabolites, were detected. Based on *in silico* analyses and experimental studies, such as transposon mutagenesis and directed gene deletion studies, the pathways of rubber and GP degradation were proposed and the relationship between both pathways was unraveled. The genes involved include, *inter alia*, genes participating in cell envelope synthesis (long-chain-fatty-acid-AMP ligase and arabinofuranosyltransferase), β -oxidation (α -methylacyl-coenzyme A [α -methylacyl-CoA] racemase), propionate catabolism (acyl-CoA carboxylase), gluconeogenesis (phosphoenolpyruvate carboxykinase), and transmembrane substrate uptake (Mce [mammalian cell entry] transporter). This study not only improves our insights into the mechanism of microbial degradation of rubber and GP but also expands our knowledge of the genus *Nocardia* regarding metabolic diversity.

Polyisoprene is one of the most important polymeric materials in our society because of its special properties for a wide range of applications. The *cis*-isomer of polyisoprene [poly(*cis*-1,4-isoprene)] is known as the main component of natural rubber and can be synthesized by more than 2,500 plants and some fungi (1). Due to its superior elasticity, it is used extensively to produce mobile tires, rubber hoses, latex gloves, conveyers, condoms, etc. The *trans*-isomer [poly(*trans*-1,4-isoprene)], which is the main component of gutta-percha (GP), can also be synthesized by a few plants, such as *Palaquium gutta*, *Eucommia ulmoides*, and *Couma macrocarpa*. Unlike rubber, GP is rigid at room temperature. Its enhanced insulation property and excellent resistance against microbial decomposition make it a desirable material for splints, pipes, and golf balls and for filling of tooth canals in dentistry (2). On the other hand, the wide range of applications of polyisoprenes accordingly leads to problems in waste treatment due to the large volume of produced items and their durability. This is particularly the case for rubber. For example, the global annual production of rubber increased to about 26.4 million tons (43% natural rubber and 57% synthetic rubber) in 2012, which is about 8% more than the level in 2010 (3). Incineration is the most widely used method for rubber waste treatment; however, this is not desirable because it causes a secondary pollution (CO, NO_x, and SO₂) of ambient air (4). A risk of air, water, and soil pollution also occurs during other recycling methods, such as pyrolysis (5). Therefore, it is important to find a more economic and environmentally friendly treatment method, for which microbial degradation may be competitive in the future.

In the past few decades, more than 100 rubber-degrading bacteria have been identified from different habitats (6–9). They are divided into the following two groups according to their degradation behavior (9): (i) the latex-clearing group of bacteria, which produce translucent halos on rubber latex

overlay plates (10); and (ii) the adhesive growth group of bacteria, which require direct contact of the cells with rubber substances for growth. Through the identification of degradation intermediates (11), the random mutagenesis of efficient degrading bacteria, and genomic analysis (12, 13), a complete rubber degradation pathway could be depicted (14). In contrast, the knowledge about GP biodegradation is quite limited. In the effort to screen GP-degrading microorganisms, it was interesting to find that GP-degrading ability always occurs in combination with the ability to degrade rubber, but not *vice versa*. The only six known GP-degrading bacterial isolates (*Nocardia nova* SH22a, L1b, SEI2b, and SEI5a, *Nocardia jiangxiensis* SM1, and *Nocardia takedensis* WE30) were also found to be efficient decomposers of rubber (2). In our previous study (15), the synthetic and natural rubber-degrading strain *N. nova* SH22a was chosen as a model microorganism to investigate the GP degradation mechanism and the potential relationship between GP and rubber degradation by use of the strategy of transposon mutagenesis. The growth of most degradation-defective mutants was affected on both polyisoprenoids (GP and rubber), indicating that the GP and rubber degradation pathways are quite similar and share many common steps. The determination of transposon insertion loci suggested that en-

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TABLE 1 Strains, plasmids, and oligonucleotides used in this study

Strain, plasmid, or oligonucleotide	Characteristic, description, or sequence (5'–3') ^a	Reference, source, or restriction site
Strains		
<i>N. nova</i> SH22a	GP- and rubber-degrading wild type	2
<i>N. nova</i> SH22a SE3-4-18	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a SE13-5-5	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a SE16-4-35	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a SE18-1-3	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a SE18-8-1	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a OC11-7-29	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a OC20-2-42	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a SE10-4-21	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a OC14-14-5	Transposon-induced mutant, GP-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a SE9-8-16	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a OC19-4-17	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a OC22-3-17	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a OC26-3-28	Transposon-induced mutant, GP- and rubber-degradation defective, Apr ^r	This study
<i>N. nova</i> SH22a $\Delta fadD32_{SH22a}$	<i>fadD32_{SH22a}</i> deletion mutant, GP- and rubber-degradation defective, Km ^r	This study
<i>N. nova</i> SH22a Δpck	<i>pck</i> deletion mutant, GP- and rubber-degradation defective, Km ^r	This study
<i>E. coli</i> XL10-Gold	Tet ^r $\Delta(mcrA)183 \Delta(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac$ Hte [F ⁺ <i>proAB lacI</i> ^q Z Δ M15 Tn10 (Tet ^r) Amy Cam ^r]	Agilent Technologies
Plasmids		
pMA5096	Contains transposon Tn5096, Amp ^r Apr ^r	12
pSK Ω Km	Kanamycin resistance cassette-containing plasmid	Strain SK07729 ^b
pJET1.2/blunt	Blunt-end cloning vector, Amp ^r	Thermo Fisher Scientific
pJET::FAALKN	Used for <i>fadD32_{SH22a}</i> deletion experiment, Amp ^r	This study
pJET::FAALKN::Km	Used for <i>fadD32_{SH22a}</i> deletion experiment, Amp ^r Km ^r	This study
pJET::PCKKN	Used for <i>pck</i> deletion experiment, Amp ^r	This study
pJET::PCKKN::Km	Used for <i>pck</i> deletion experiment, Amp ^r Km ^r	This study
pNV18.1	<i>E. coli-Nocardia</i> shuttle vector, Km ^r Neo ^r	96
pNV18.1:: <i>fadD32_{SH22a}</i>	Used for complementation experiment, Km ^r Neo ^r	This study
pNV18.1:: <i>pck</i>	Used for complementation experiment, Km ^r Neo ^r	This study
Oligonucleotides		
FAAL- <i>kn1</i>	TGAGGGCAACCAGCAGGAG	
FAAL- <i>kn2</i>	CAGAACTCGCCGGGCGCACGAT <u>CCCGGGGGCCGCTGATACCTGTGAG</u>	SmaI
FAAL- <i>kn3</i>	CTCACAGGTATCAGCGCC <u>CCCGGGATCGTGCCCGGCGAGTTCTG</u>	SmaI
FAAL- <i>kn4</i>	GATGAAGACGCCGACCCGCTC	
PCK- <i>kn1</i>	AAACATATGGTGGATCTGACGCACGAGG	NdeI
PCK- <i>kn2</i>	CGTCCGAAAAAGCCTATCCAGGTCGACGAAAACTCTCTGAGATGAGCG	HincII
PCK- <i>kn3</i>	CGCTCATCTCAGGAGAGTTTTCTCGACCTGGGATAGGCTTTTTTCGACG	HincII
PCK- <i>kn4</i>	AAACATATGGAACCGCACCCACACCTC	NdeI
FAAL- <i>exp1</i>	GAAGACCATCGAGAAGGTG	
FAAL- <i>exp2</i>	GATGCTGATACACGATGGTC	
PCK- <i>exp2</i>	AGTCCGGTCATGCCACAC	

^a Amp^r, ampicillin resistance; Apr^r, apramycin resistance; Cam^r, chloramphenicol resistance; Gm^r, gentamicin resistance; Km^r, kanamycin resistance; Neo^r, neomycin resistance; Tet^r, tetracycline resistance. Underlining in the sequences indicates the restriction endonuclease sites for DNA cloning.

^b From the strain collection of our lab.

zymes participating in oxidoreduction reactions, β -oxidation, and the synthesis of complex cell envelope lipids are involved in the degradation processes.

In the present study, the identification of enzymes involved in GP and/or rubber degradation was continued, and the complete genome of SH22a was sequenced. Based on the experimental results and an *in silico* genome analysis, a more detailed polyisoprene degradation pathway (for both the *cis*-isomer and the *trans*-isomer) is illustrated. Moreover, the elaboration of the *N. nova* genome is also quite helpful for a better understanding of the genus *Nocardia* with respect to the anabolism of secondary inter-

mediates and the catabolism of other organic environmental pollutants (16).

MATERIALS AND METHODS

Bacterial strains, plasmids, and cultivation conditions. Bacterial strains and plasmids used in the present study are listed in Table 1. If not stated otherwise, *N. nova* was grown in CASO broth (Carl Roth, Germany) at 30°C, whereas *Escherichia coli* was grown in lysogeny broth (LB) at 37°C. In growth experiments, *N. nova* was grown in mineral salts medium (MSM) (17) at 30°C, and carbon sources were added as indicated in the text. Liquid cultures were grown in Erlenmeyer flasks, which were incu-

bated on a horizontal rotary shaker at 140 rpm. Solid media were prepared by adding 1.6% (wt/vol) agar-agar. Antibiotics were used as described by Sambrook et al. (18) for *E. coli* strains, whereas 50 µg of kanamycin/ml or 50 µg of apramycin/ml was applied for *N. nova* strains.

Growth of *N. nova* on GP and rubber. Growth experiments with *N. nova* strains were performed on solid and in liquid MSM with GP or rubber as the sole carbon and energy source. For this purpose, synthetic poly(*trans*-1,4-isoprene) (99%; Sigma-Aldrich) or poly(*cis*-1,4-isoprene) (97%; Sigma-Aldrich) was used, respectively. MSM-GP and MSM-rubber sandwich agar plates were used as solid media and were prepared according to a previously described method (12). *Nocardia* cells were inoculated by pushing toothpicks through the top MSM agar layer into the GP or rubber film to provide cells with direct contact to the polyisoprenes. For growth in liquid MSM, *trans*- or *cis*-polyisoprene was cryomilled to a defined grain size (≤ 500 µm). Strains were cultivated in liquid MSM containing 0.3% (wt/vol; either type) polymer grain.

Transposon mutagenesis and screening for mutants defective in rubber or GP degradation. The Tn5096-containing suicide plasmid pMA5096 was employed for the transposon mutagenesis of *N. nova* SH22a. Details about the transposition procedure were described previously (15). Transposon-induced transformants were screened on MSM-rubber and MSM-GP sandwich plates to identify mutants showing defective phenotypes in rubber or GP utilization. Auxotrophic mutants were excluded by using glucose as the sole carbon and energy source, as described previously (15).

DNA extraction and manipulation. Total DNA of *N. nova* was isolated according to the DNeasy Blood & Tissue kit protocol (Qiagen, Germany) or by a previously described method (19). Plasmid DNA was isolated from crude cell lysates by the alkaline extraction method (20). Cells were broken in lysis buffer with additional lysozyme (1 mg/ml) at 37°C for 1 h when plasmids were prepared from *N. nova*. Other genetic procedures and molecular manipulations were performed according to standard protocols (18). The *Pfx* DNA polymerase used for DNA amplification by PCR was obtained from Invitrogen. T4 DNA ligase and restriction enzymes were purchased from Thermo Fisher Scientific. Primers used in this study were synthesized by Eurofins MWG (Ebersberg, Germany) and are listed in Table 1.

Construction and complementation of *N. nova* deletion mutants. In order to delete the *fadD32*_{SH22a} gene (NONO_C01500), the flanking regions upstream (flankA; 1.15 kb) and downstream (flankB; 1.0 kb) of *fadD32*_{SH22a} were amplified by using *Pfx* polymerase and employing the primer pairs FAAL-kn3/FAAL-kn4 and FAAL-kn3/FAAL-kn4, with total DNA of *N. nova* SH22a as the PCR template. The resulting fragments (flankA and flankB) were linked to a fused fragment (flankA-B; 2.15 kb) via fusion PCR (21). The flankA-B fragment was subsequently ligated into the cloning vector pJET1.2/blunt (Thermo Fisher Scientific), yielding pJET::FAALKN. A 1.0-kb kanamycin resistance cassette (Km), which was excised from pSKΩKm by using SmaI, was cloned into the single SmaI site between flankA and flankB, yielding pJET::FAALKN::Km. A 3.15-kb linear fragment (flankA-Km-flankB) was excised from pJET::FAALKN::Km by use of Kpn2I and XbaI sites (located in pJET1.2/blunt) and subsequently transferred into competent SH22a cells by electroporation (15) for homologous recombination on kanamycin plates. Genotypes of recombinants were confirmed by PCR, and the positive mutant *N. nova* SH22a Δ *fadD32*_{SH22a} was obtained. A similar procedure employing primer pairs PCK-kn1/PCK-kn2 and PCK-kn3/PCK-kn4 was used to construct the *pck* (NONO_c74450) deletion mutant *N. nova* SH22a Δ *pck*. The linear fragment used for homologous recombination was excised from plasmid pJET::PCKKN::Km by use of NdeI.

For complementation experiments, a 3.07-kb fragment of *fadD32*_{SH22a} (primer pair FAAL-exp1/FAAL-exp2) and a 3.1-kb fragment of *pck* (primer pair PCK-kn1/PCK-exp2), each containing the native promoter, were amplified from total DNA of SH22a. The fragments were cloned into the HincII site of the vector pNV18.1, yielding pNV18.1::*fadD32*_{SH22a} and pNV18.1::*pck*. The nucleotide sequences of *fadD32*_{SH22a} and *pck* were

confirmed by sequencing. The resulting plasmids were then electrotransformed into the Δ *fadD32*_{SH22a} mutant and the Δ *pck* mutant, respectively, for complementation.

Genome sequencing, assembly, and gap closure. Genomic DNA of *N. nova* SH22a was isolated by use of a DNeasy Blood & Tissue kit (Qiagen, Germany). The extracted DNA was used to generate 454 shotgun, paired-end, and Illumina shotgun libraries according to the protocols of the manufacturers (Roche and Illumina). The libraries were sequenced using a 454 GS-FLX system (Titanium GS70 chemistry; Roche Life Sciences, Mannheim, Germany) and a Genome Analyzer II system (Illumina, San Diego, CA). The sequencing of the 454 shotgun libraries resulted in 271,866 reads, 27,749 of which were paired. Sequencing of the Illumina paired-end library resulted in 3,608,918 reads with a length of 112 bp. Assembly of the reads by use of Roche Newbler assembly software 2.6 for scaffolding and MIRA software (22) resulted in 17 scaffolds with 111 contigs, exhibiting an average coverage of 42.23. The remaining gaps were closed with PCR-based techniques and Sanger sequencing of the products, employing the Gap4 (v.4.11) software of the Staden package (23).

Genome annotation and analysis and comparative genomics. Coding sequences were automatically predicted by YACOP (24), using the open reading frame (ORF) finders Glimmer, Critica, and Z-Curve, while RNAmmer (25) and tRNAscan (26) were used for identification of rRNA and tRNA genes, respectively. Functional annotation of the 7,583 predicted protein-encoding genes was initially carried out with the IMG/ER (Integrated Microbial Genomes/Expert Review, Joint Genome Institute, U.S. Department of Energy) system (27) and then manually judged on the annotation platform Artemis (28) by using criteria such as GC frame plot analysis, the presence of a ribosome binding site, and comparison with sequences in public databases, i.e., Swiss-Prot, TrEMBL, the nonredundant database of the NCBI, KEGG, COG, Pfam, and InterProScan, as well as the sequence analysis servers of SignalP v4.1 (29), TatP v1.0 (30), and TMHMM v2.0. Comparative genomics was done by using the abundance profile search and phylogenetic profiler functions integrated into the IMG/ER system, employing default settings, as well as the BLAST package of NCBI.

Nucleotide sequence accession number. The sequence of the complete genome of *N. nova* SH22a is available under NCBI accession number CP006850.

RESULTS AND DISCUSSION

General features of *N. nova* SH22a genome. The complete genome of *N. nova* SH22a comprises a circular chromosome of 8,348,532 bp with a G+C content of 67.77% (Table 2). Unlike the genome of *Nocardia farcinica* IFM 10152 (31), the SH22a genome does not contain any plasmids. The SH22a genome contains 7,583 predicted protein-encoding genes, among which 5,494 (72.45%) could be assigned a putative function. In addition, 49 tRNA genes and three copies of the 16S-23S-5S rRNA gene were found. Five pseudogenes were detected in the genome of *N. nova* SH22a.

A comparison of the SH22a genome with the other three fully sequenced genomes of the genus *Nocardia* (*N. farcinica* IFM 10152 [31], *Nocardia cyriacigeorgica* GUH-2 [32], and *Nocardia brasiliensis* HUJEG-1 [33]) was carried out. These analyses revealed that in comparison to the predicted proteomes of the other *Nocardia* strains, many protein families of SH22a which might be involved in the degradation of xenobiotics, other hardly degradable compounds, and polymers are represented at higher percentages. These include, *inter alia*, aldehyde dehydrogenase family proteins and enzymes involved in β -oxidation, which might be a reason for the rubber- and GP-degrading phenotype (as discussed below), but also a large number of transporters, which might be involved in the uptake of the hardly degradable compounds (or degradation intermediates). The latter include, in particular, 14 ATP-de-

TABLE 2 General features of the genome of *N. nova* SH22a and a comparison of fully sequenced *Nocardia* genomes

Characteristic	Value			
	<i>N. nova</i> SH22a ^a	<i>N. farcinica</i> IFM10152 ^a (31)	<i>N. cyriaciageorgica</i> GUH-2 ^a (97)	<i>N. brasiliensis</i> HUJEG-1 ^b (33)
Genome size (bp)	8,348,532	6,292,344	6,194,645	9,436,348
No. of circular chromosomes	1	1	1	1
No. of plasmids	0	2	0	0
GC content (%)	67.77	70.69	68.37	68.05
Coding density (%)	89.5	90.4	87.1	87.7
No. of protein-encoding genes	7,583	5,936	5,491	8,414
With function prediction	5,494	3,039	3,065	2,669
Without function prediction	2,089	2,897	2,426	5,745
No. of rRNA genes	9	9	9	9
No. of tRNA genes	49	54	49	51

^a Data were taken from the Integrated Microbial Genomes (IMG) database of the Department of Energy, Joint Genome Institute.

^b Data are from reference 33.

pendent transporters of the mammalian cell entry (Mce) type, among which some were already shown to be involved in the transport of lipophilic substances (34, 35) and degradation products of inert polymers (14) in other microorganisms. The number of Mce gene clusters in SH22a is the largest found in a genome so far. Among the four sequenced *Nocardia* genomes, a unique feature of SH22a is the occurrence of genes for many enzymes that might be responsible for the degradation of aromatic compounds, such as ring-cleavage dioxygenases (e.g., ring-cleavage extradiol dioxygenases [NONO_c21130, NONO_c37930, NONO_c50010, and NONO_c50140] and Rieske [2Fe-2S]/ring-hydroxylating alpha subunit domain-containing proteins [NONO_c42810, NONO_c43140, and NONO_c50820]). Furthermore, the genome of SH22a carries genes for a complete set of enzymes required for the Calvin-Benson-Bassham cycle, which are also present in *N. cyriaciageorgica* GUH-2 but not in the genomes of *N. brasiliensis* HUJEG-1 and *N. farcinica* IFM 10152. Moreover, the genes for two putative [NiFe] hydrogenases (NONO_c30160/NONO_c30170 and NONO_c49680/NONO_c49690) were found exclusively in SH22a. However, growth experiments under an H₂-O₂-CO₂ (8:1:1 [vol/vol/vol]) atmosphere demonstrated that strain SH22a was not able to grow autotrophically, whereas the positive control, *Ralstonia eutropha* H16, had that capability (data not shown).

GP and rubber catabolism of *N. nova* SH22a. In a previous study, we constructed a Tn5096-based transposon mutagenesis library of *N. nova* SH22a to investigate the mechanism of microbial GP and rubber degradation. This yielded 76 transposon-induced mutants stably showing defective growth on GP and/or rubber but not on glucose or complex media (15). Further determination of the transposition loci of these mutants and *in silico* analysis of the SH22a genome led to the characterization of a series of novel enzymes/proteins involved in the degradation of GP and rubber (Fig. 1). The related genes and their possible functions are discussed in detail below.

Adhesion of nocardial cells to GP and rubber substrates. Some actinobacterial genera belonging to the order *Corynebacteriales*, such as *Nocardia*, *Rhodococcus*, *Gordonia*, and *Mycobacterium*, are able to colonize the surfaces of various hydrophobic substances and utilize them as the sole carbon and energy source

(36–39). This adhesive growth behavior is thought to be due to the hydrophobic interaction between the hydrophobic cell surface and the substance. Therefore, it is expected that any interruption of the integrity and composition of cell envelopes will significantly affect the adhesion property.

Mycolic acids, which are the major constituent of cell envelopes of the *Corynebacteriales* (40) and are important for many cell surface properties, such as virulence (41) and low permeability (42), also play a crucial role in the high affinity of cells for numerous hydrophobic substances (43). The mechanism of synthesis, modification, transfer, and assembly of mycolic acids was recently demonstrated in detail, and the related genes were identified in *Mycobacterium* and *Corynebacterium* (44–46). *fadD32-pks13-accD4* is a well-studied gene cluster that is involved in the terminal steps of mycolic acid synthesis (46). This cluster and its adjacent genes compose a conserved region. It is widely found in many other *Corynebacteriales* species, including the three fully sequenced *Nocardia* strains (*N. farcinica* IFM 10152 [nfa1880 to nfa1900], *N. cyriaciageorgica* GUH-2 [NOCYR_0144 to NOCYR_0146], and *N. brasiliensis* HUJEG-1 [O3I_20420 to O3I_20410]). As expected, homologues of *fadD32*, *pks13*, and *accD4* were detected in the genome of SH22a and were predicted to encode a long-chain-fatty-acid-AMP ligase (FadD32_{SH22a}; NONO_c01500), a polyketide synthase (PKS)-type condensase (NONO_c01510), and an acyl-coenzyme A (acyl-CoA) carboxylase beta subunit (NONO_c01520), respectively.

In the genomes of seven transposon-induced mutants (SE3-4-18, SE13-5-5, SE16-4-35, SE18-1-3, SE18-8-1, OC11-7-29, and OC20-2-42) (Table 1) which were defective in both GP and rubber degradation, the Tn5096 transposon-containing plasmid pMA5096 was found to interrupt the same gene, *fadD32*_{SH22a}, by either inserting into different sites of this gene or replacing a chromosomal region concerning this gene (Fig. 2A). These results indicate a relevancy between mycolic acids and GP and rubber degradation. However, in order to confirm the molecular basis of the phenotypic deficiency in more detail, for example, to exclude the polar effect on the downstream genes *pks13*_{SH22a} and *accD4*_{SH22a} or the influence on the upstream gene (NONO_c01490), deletion and complementation experiments were performed for *fadD32*_{SH22a}. The deletion mutant *N. nova* SH22a

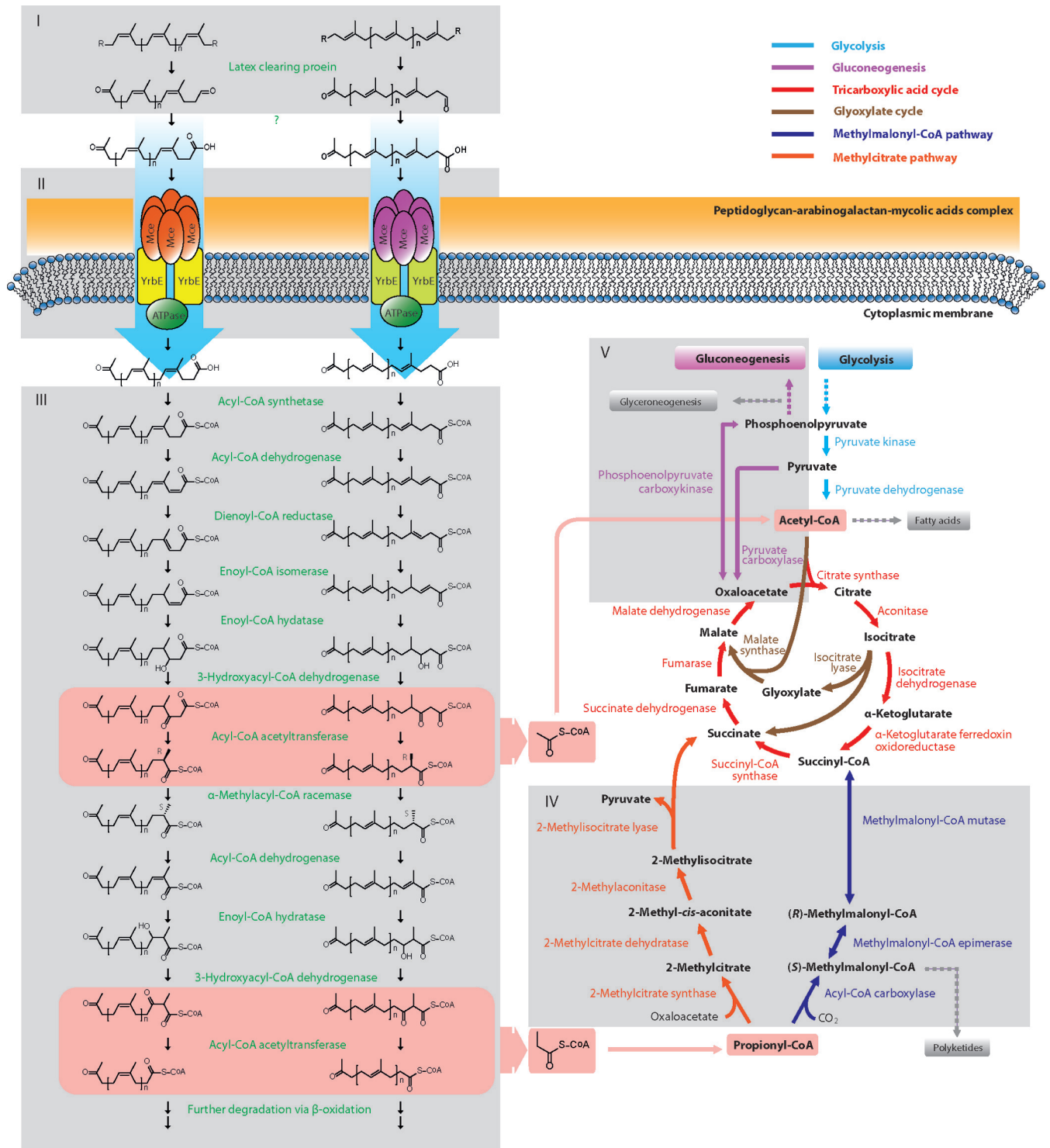


FIG 1 Proposed metabolic pathway of polyisoprene degradation in *N. nova* SH22a. The six gray blocks labeled by roman numbers represent the main parts of the proposed degradation pathways. I, initial oxidative cleavage of polyisoprene chains (indirect evidence from other studies [13, 14, 51]); II, import of oligoisoprenes (direct evidence from transposon-induced mutants of Mce in strain SH22a [this work] and also in the closely related strain *G. polyisoprenivorans* VH2 [14]); III, β -oxidation (direct evidence from transposon-induced mutants, deletion mutants, and complementation experiments for α -methylacyl-CoA racemase in strain SH22a [15], and also indirect evidence from other studies [62, 98]); IV, propionyl-CoA metabolism (direct evidence from transposon-induced mutants in strain SH22a [this work]); V, gluconeogenesis (direct evidence from transposon-induced mutants, deletion mutants, and complementation experiments for phosphoenolpyruvate carboxykinase [this work]). The two pink bars show the steps alternately releasing degradation products (acetyl-CoA and propionyl-CoA) of β -oxidation.

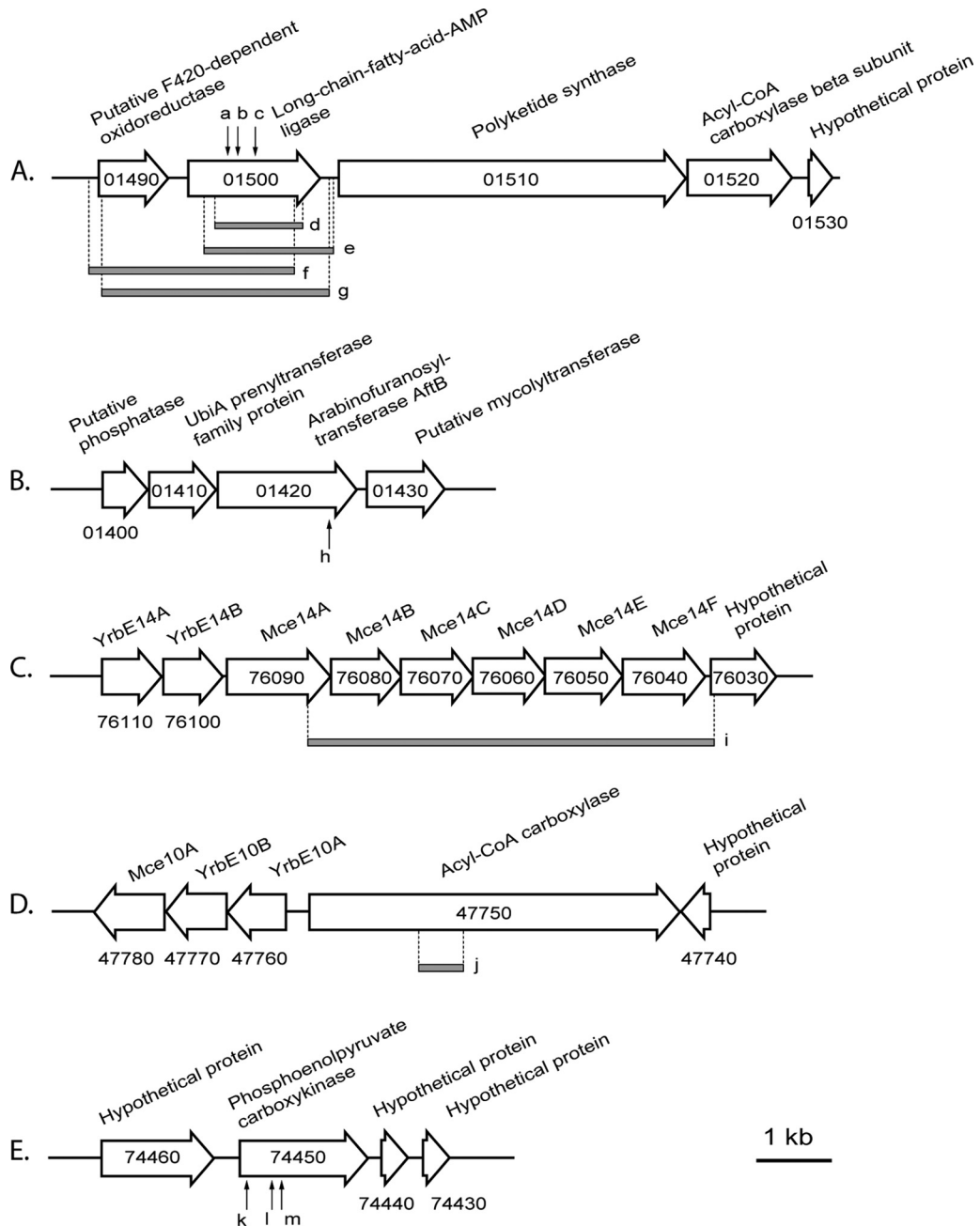


FIG 2 Visualization of pMA5096 insertions in the genome of *N. nova* SH22a transposon-induced mutants and identification of genes adjacent to the transposon loci. Arrows indicate the insertion sites of pMA5096, and the gray bars represent the missing chromosomal regions caused by pMA5096 transposition. Mutants: a, OC11-7-29; b, SE3-4-18; c, SE16-4-35; d, SE18-8-1; e, SE13-5-5; f, OC20-2-42; g, SE18-1-3; h, SE10-4-21; i, OC14-14-5; j, SE9-8-16; k, OC26-3-28; l, OC22-3-17; m, OC19-4-17. The prefix “NONO_c” of locus tags is omitted in the diagram. The lengths of open arrows showing the genes indicate the proportional lengths of transcription of the corresponding genes. Annotations of genes are given above the corresponding arrows.

Δ *fadD32*_{SH22a}, in which *fadD32*_{SH22a} was replaced by a 1.0-kb kanamycin resistance cassette, was generated by homologous recombination. Like the transposon-induced mutants, this deletion mutant showed a growth deficiency on *trans*- and *cis*-polyisoprene, but not glucose, as the sole carbon and energy source (Fig. 3). Moreover, when phytol (0.5% [vol/vol]), a highly hydrophobic, oil-like substance, was used in the liquid MSM for growth, the deletion mutant did not aggregate tightly on the oil drops, whereas the wild type did. When plasmid pNV18.1::*fadD32*_{SH22a} harbor-

ing the native *fadD32*_{SH22a} gene, was transferred into the Δ *fadD32*_{SH22a} strain, the deficiency in GP and rubber degradation was completely eliminated in the resulting recombinant mutant (Fig. 3). In order to observe phenotypes under the same growth conditions, no antibiotics were added to the medium. Accordingly, plasmids were isolated from the complemented mutant after 70 days of cultivation in liquid MSM medium containing *cis*- or *trans*-polyisoprene and retransformed into *E. coli* cells for proliferation. The same restriction patterns of these plasmids con-

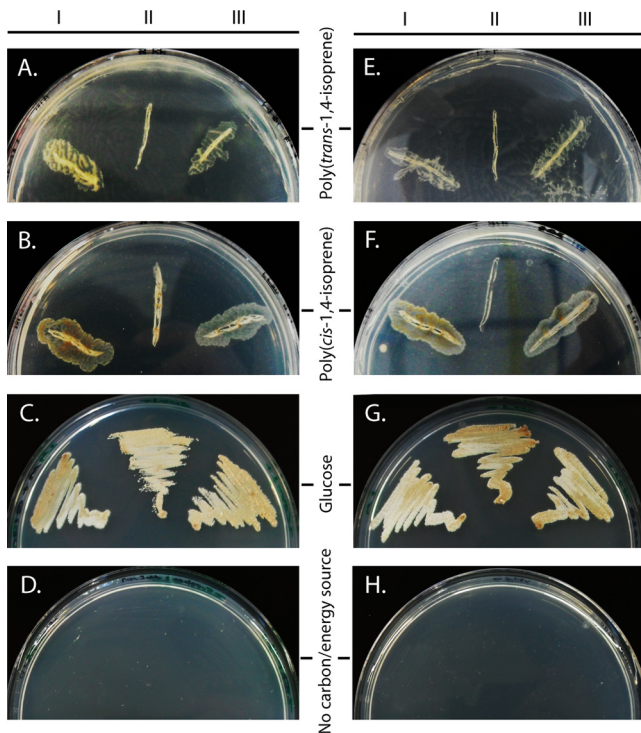


FIG 3 Growth of *N. nova* SH22a on solid MSM medium in the presence or absence of poly(*trans*-1,4-isoprene), poly(*cis*-1,4-isoprene), or glucose as the sole source of carbon and energy. I, *N. nova* SH22a wild-type strain; II, *N. nova* SH22a Δ *fadD32*_{SH22a} (A to D) or *N. nova* SH22a Δ *pck* (E to H); III, *N. nova* SH22a Δ *fadD32*_{SH22a}(pNV18.1::*fadD32*_{SH22a}) (A to D) or *N. nova* SH22a Δ *pck*(pNV18.1::*pck*) (E to H).

firmated that they were derived from the original parents and could replicate stably in *N. nova* SH22a even under conditions without selective pressure. These results solidly confirmed that *fadD32*_{SH22a} is an essential gene for SH22a to degrade both GP and rubber.

The long-chain-fatty-acid-AMP ligase FadD32 is known to catalyze the activation of meromycolic acids, which are precursors of mycolic acids (47). Portevin et al. (48) reported that the Δ *fadD32::km* disruption mutant of *Corynebacterium glutamicum* was devoid of mycolic acids. It can be expected that this also happens in the Δ *fadD32*_{SH22a} mutant, because FadD_{SH22a} exhibits a high similarity toward FadD32 of *C. glutamicum* (42% identity). In addition, the organization of the whole cluster (*fadD32-pks13-accD4*) is highly conserved in *Corynebacteriales* species. Since it is known that mycolic acids are important for the adhesive growth of mycolic acid-containing actinobacteria on hydrophobic substances (43), the prevention of mycolic acid synthesis in SH22a may thereby weaken the affinity of the cell surface for hydrophobic substances, e.g., GP, rubber, and phytol. As a result, a deficiency in the utilization of GP and rubber was exhibited. On the other hand, the modified cell envelopes might also hamper the assembly of certain macromolecule-specific transporters in the cytoplasmic membrane, which in turn would affect the uptake of GP and rubber substrates.

Furthermore, another cell wall synthesis gene was identified in the GP and rubber degradation-defective mutant SE10-4-21. pMA5096 was found to insert into the 3'-terminal region of

NONO_c01420 (Table 1; Fig. 2B). This ORF is located in an "ancient conserved region" (NONO_c01400 to NONO_c01520) which is known to be involved in cell envelope synthesis and also includes the *fadD32-pks13-accD4* cluster of CMN (*Corynebacteria*, *Mycobacteria*, and *Nocardia*) group actinobacteria (49). NONO_c01420 was annotated as an arabinofuranosyltransferase gene (51% amino acid sequence identity to AftB of *Mycobacterium tuberculosis*) whose product catalyzes the β (1 \rightarrow 2) linkage of terminal arabinofuranosyl residues to the arabinan skeleton in arabinogalactan biosynthesis (50). Since the terminal arabinofuranosyl residues subsequently provide partial sites for mycolic acid esterification, this step is important for the assembly of mycolic acids. Seidel et al. found that deletion of the *aftB* gene in *C. glutamicum* directly resulted in a decreased abundance of cell wall-bound mycolic acids (50). Therefore, it is expected that the disruption of NONO_c01420 led to an incomplete assembly of cell walls, which also, in turn, affected the required adhesion between nocardial cells and substrates.

Initial oxidation. In order to serve as substrates that can be metabolized by bacterial cells, the high-molecular-weight GP or rubber polymers should first be degraded to low-molecular-weight oligomers outside cells (Fig. 1). For *cis*-isomers, an endocleavage mechanism is adopted by rubber-degrading bacteria for this conversion (6, 51). Previous studies have shown that this is an O₂-dependent process. Shortened rubber oligomers with aldehyde and keto groups at their termini were generated after the oxidative cleavage of double bonds of poly(*cis*-1,4-isoprene) (6, 51). Before now, two enzymes (RoxA [rubber oxygenase] [52] and Lcp [latex-clearing protein] [13]) involved in the initial cleavage step had been identified. In contrast to RoxA, which occurs in a few Gram-negative bacteria, such as *Xanthomonas* sp. 35Y (rubber-degrading bacterium) and *Haliangium ochraceum* (non-rubber-degrading bacterium) (53), Lcp is more widely distributed in rubber-degrading actinobacteria, such as *Streptomyces* sp. K30 (13), *N. farcinica* (51), *Actinoplanes missouriensis* (8), *Gordonia westfalica*, and *Gordonia polyisoprenivorans* (54). It was observed that this protein is not only required for latex-clear-zone formation but also essential for the growth of adhesive-group bacteria on rubber (14). In the present study, an ORF (NONO_c40940) coding for an LCP protein was identified in the genome of SH22a. The corresponding translation product exhibits high sequence identity to the well-studied LCPs (52% identity to LCP of *Streptomyces* sp. K30, 64% identity to LCP of *N. farcinica* E1, and 63% and 72% identity to LCP1 and LCP2 of *G. polyisoprenivorans* VH2, respectively) (13, 14, 51). Considering that the GP degradation pathway is quite similar to that of rubber degradation, we speculate that LCPs also play an essential role in GP degradation.

Since aldehydes are unstable toward oxidation and usually toxic to bacterial cells, the aldehyde groups of GP and rubber oxidative cleavage products are supposed to be oxidized immediately to the corresponding acids outside cells. Rose et al. speculated that *oxiAB*, which is located directly downstream of *lcp* and predicted to encode an oxidoreductase complex, might be responsible for this reaction in *Streptomyces* sp. K30 (13). However, such a genetic organization pattern of *lcp-oxiAB* or the deduced proteins showing similarities to those encoded by *oxiAB* was not observed in most known rubber-degrading bacteria, including SH22a. Therefore, there must be another enzyme catalyzing this reaction. The candidate could be one of the numerous oxygenases encoded by the SH22a genome. However, an inser-

tion of pMA5096 in one of the predicted oxygenase-encoding genes was not detected in the transposon-induced mutants. This might be due to the presence of two (or more) oxygenases catalyzing this step.

Mce transporter. Although *mce* clusters, which have identical structures consisting of two *yrbE* genes (*yrbEA* and *yrbEB*), encoding integral membrane proteins, followed by six *mce* genes (*mceA* to *mceF*), encoding exported or membrane-tethered proteins, were first identified due to their critical role in bacterial invasion and survival in mammalian cells (55), not all bacteria possessing *mce* clusters are found as pathogens (such as *Mycobacterium smegmatis* and *Streptomyces coelicolor*). This indicates that the products of *mce* clusters have physiological functions other than acting as virulence factors, e.g., acting as novel ABC uptake transporters (56). This speculation was soon proved by showing that the Mce4 system of *Rhodococcus jostii* RHA1 functions as a steroid uptake transporter (34).

Regarding GP and rubber degradation, the cleavage products have to be transported into cells for further metabolism. A preliminary hint, obtained from the rubber-degrading bacterium *G. polyisoprenivorans* VH2 (14), suggested that these transporters are probably encoded by *mce* clusters (Fig. 1). During our identification of genes involved in GP and/or rubber degradation, we detected a mutant, OC14-14-5 (Table 1; Fig. 2C), in which five complete *mce* genes (NONO_c76080 to NONO_c76040) within the *mce14* cluster were missing because of the insertion of pMA5096. Interestingly, the disruption of *mce14* specifically led to a GP degradation-defective phenotype, while the growth of this mutant on rubber was not affected at all. When the protein sequences encoded by the *mce14* cluster were compared with those for *mce* clusters of *G. polyisoprenivorans* VH2, the best match was for one (GPOL_c27400 to GPOL_c27470) showing an involvement in rubber degradation (14). However, when this analysis was performed in reverse, this gordonial *mce* cluster did not show highest similarity exclusively to the *mce14* cluster of SH22a. Therefore, we suppose that the Mce14 transporter system of SH22a is specifically involved in GP degradation and that the uptake of rubber substrates is transported by another Mce transporter system(s). An important characteristic of ABC-type transporters is the requirement of an ATPase to provide energy for substrate translocation. Mkl family ATPases, whose coding genes were found to be proximal to certain *mce* clusters in nonmycobacterial actinomycetes or separated from any *mce* cluster in *Mycobacterium*, were thought to be the enzymes involved (34, 56, 57). The genes for three Mkl ATPases (NONO_c31130, NONO_c50930, and NONO_c69480) were detected in the genome of SH22a. NONO_c50930 and NONO_c69480 are located immediately upstream of *mce* clusters, i.e., *mce11* (NONO_c50920 to NONO_c50850) and *mce13* (NONO_c69470 to NONO_c69400), respectively, whereas NONO_c31130 is separate from *mce* clusters and located near a putative transcriptional regulator gene (NONO_c31120).

β -Oxidation. β -Oxidation is known as a basic and iterative biological process catalyzing the cleavage of two carbon units each cycle from long-chain hydrocarbons. It is not only required for the breakdown of fatty acids in lipid catabolism of prokaryotic and eukaryotic organisms but also important for the complete decomposition of a variety of aliphatic and aromatic hydrocarbons in many bacteria (39, 58–61). In recent studies, an involvement of β -oxidation enzymes (e.g., acyl-CoA synthetase, thiolase, and α -methylacyl-CoA racemase [MCR]) in the degradation of rub-

ber and/or GP was observed in strain SH22a (15) and another rubber-degrading bacterium, *G. polyisoprenivorans* VH2 (14, 62), which strongly indicates that β -oxidation is also employed by these bacteria for the further metabolism of polyisoprenes (Fig. 1). In the present study, we identified 79 putative acyl-CoA synthetases, 81 putative acyl-CoA dehydrogenases, 1 putative 2,4-dienoyl-CoA reductase, 64 putative enoyl-CoA hydratases/isomerases, 10 putative 3-hydroxyacyl-CoA dehydrogenases, 24 putative thiolases, and 5 putative MCRs that may participate in β -oxidation encoded in the genome of strain SH22a (see the supplemental material). The large number of enzyme candidates for each step may explain why only one β -oxidation-related enzyme (MCR) was identified from transposon-induced mutants of SH22a (15), since two or more homologues may commonly catalyze the same degradation step.

Central metabolism related to GP and rubber degradation. Although GP and rubber are macromolecular compounds, their degradation products will undoubtedly enter the central metabolism for generating energy and providing a carbon skeleton for synthesizing other essential molecules. Genes for central metabolism, such as those for the Embden-Meyerhof-Parnas pathway (glycolysis), the pentose phosphate pathway, the tricarboxylic acid (TCA) cycle, the glyoxylate cycle, and the gluconeogenesis pathway, were detected in the genome of SH22a.

Results of our previous study (15) as well as the present study support the hypothesis that GP degradation undergoes a pathway similar to that of rubber degradation. This process is quite similar to that of polyunsaturated fatty acid degradation and generates acetyl-CoA and propionyl-CoA (Fig. 1) as degradation products. Whereas acetyl-CoA directly enters the TCA cycle and/or the glyoxylate bypass, propionyl-CoA is coupled with the central metabolism via two pathways probably occurring in SH22a, the methylcitrate pathway and the methylmalonyl-CoA pathway. The former pathway mediates the oxidation of propionyl-CoA to pyruvate (63, 64). Two *prpDBC* clusters (NONO_c35960 to NONO_c35980 and NONO_c62610 to NONO_c62590), encoding the main enzymes (2-methylcitrate dehydratase, 2-methylisocitrate lyase, and 2-methylcitrate synthase) of this pathway, were deduced from the SH22a genome. The methylmalonyl-CoA pathway catalyzes the conversion of propionyl-CoA to succinyl-CoA by the enzymes acyl-CoA carboxylase (ACCase), methylmalonyl-CoA epimerase, and methylmalonyl-CoA mutase and is known as an important route for propionate metabolism in some microorganisms (65, 66). More importantly, this pathway enables the production of (S)-methylmalonyl-CoA, which serves as a key precursor for the synthesis of many polyketides (67, 68). While only one copy each of the genes encoding methylmalonyl-CoA epimerase (NONO_c12700) and methylmalonyl-CoA mutase (NONO_c41040/NONO_c41050) was detected in the SH22a genome, ACCase was found to be encoded at different genetic loci. These include five carboxylase systems, where the α -subunit (NONO_c10710, NONO_c11500, NONO_c50500, NONO_c68560, and NONO_c71920) and β -subunit (NONO_c10730, NONO_c11450, NONO_c50510, NONO_c68570, and NONO_c71930) genes are located next (or close) to each other and four separate β -subunits (NONO_c01520, NONO_c07340, NONO_c20810, and NONO_c33050). Besides these typical prokaryotic ACCases, which comprise multiple subunits, we also detected genes for two eukaryotic-type ACCases (NONO_c40170 and NONO_c47750), which are large, multidomain proteins con-

taining a biotin carboxylase domain, biotin carboxyl carrier protein domain, and carboxyltransferase domain. Due to the fact that mycobacterial ACCases utilize both acetyl-CoA and propionyl-CoA as substrates (69), we speculated that some of these nocardial ACCases also catalyze the conversion of propionyl-CoA to methylmalonyl-CoA and are involved in GP and rubber degradation. In line with this speculation, we identified an ACCase disruption mutant, SE9-8-16, showing impaired growth on both GP and rubber. The transposon was mapped in the eukaryotic-type ACCase gene NONO_c47750 (Fig. 2D). The defective phenotype can be explained either by the catalysis of the carboxylation of propionyl-CoA by other ACCase systems or by participation of the methylcitrate pathway in propionyl-CoA metabolism. Further investigations need to be performed in the future.

In the absence of sugars as the carbon and energy source, organisms convert intermediates derived from the TCA cycle or the glyoxylate bypass to phosphoenolpyruvate (PEP), which subsequently serves as a substrate participating in many important cell processes, such as gluconeogenesis, glyceroneogenesis, amino acid synthesis, and anaplerosis of the TCA or glyoxylate cycle (70). Phosphoenolpyruvate carboxykinase (PEPCK) is the enzyme responsible for this step. It catalyzes the guanosine or adenosine mononucleotide-dependent reversible conversion of oxaloacetate and PEP. In many bacteria, the inactivation of this enzyme significantly affects the growth or survival of cells when organic acids (e.g., acetate, lactate, succinate, and malate) are used as the sole carbon and energy sources (71–74). In the present study, we observed that the PEPCK gene (*pck*; NONO_c74450) is also essential for SH22a to grow on GP and rubber. Three mutants, OC19-4-17, OC22-3-17, and OC26-3-28 (Table 1; Fig. 2E), in which *pck* was disrupted by insertions of pMA5096, completely lost the ability to grow on GP and rubber. As expected, the Δpck deletion mutant exhibited the same growth deficiency on polyisoprenes and failed to grow on acetate and pyruvate as well. This defective phenotype was restored when the Δpck mutant was transformed by a plasmid expressing native *pck* (Fig. 3), indicating that no polar effects were caused by the transposon insertions. Although a second PEPCK gene (NONO_c31000; encodes a sequence with 68% amino acid sequence identity to the former one) was detected in the genome, it seemed that the second enzyme was not active under the employed conditions, since it did not permit growth in the Δpck mutant. The physiological role of this PEPCK remains to be studied further. In the closely related species *C. glutamicum*, PEP synthesis is catalyzed exclusively by PEPCK, and the enzyme cannot be replaced functionally by an alternative route catalyzed by malic enzyme or oxaloacetate decarboxylase/PEP synthase (72, 75). The impaired growth of the Δpck mutant on GP, rubber, acetate, and pyruvate suggests that the conversion from oxaloacetate to PEP catalyzed by PEPCK is also the only route for PEP synthesis in SH22a, although genetic loci probably coding for malic enzyme (NONO_c64080) and PEP synthases (NONO_c21150, NONO_c48870, and NONO_c59210) synthases were detected.

Genes involved in metabolism of alkanes. Members of the genus *Nocardia* exhibit potent metabolic capabilities on a wide range of hydrocarbons (16). They are found to grow on linear and/or branched alkanes with different chain lengths as the sole carbon and energy source (60, 76, 77). The first and key step in alkane metabolism is the terminal or subterminal hydroxylation of alkanes to alkanols. In bacteria, this reaction is catalyzed by three categories of alkane hydroxylases: methane monooxygenase

(MMO)-like enzymes, membrane-bound nonheme iron alkane hydroxylase (AlkB or AlkM) complexes or cytochrome P450 (CYP450) family members, and other oxygenases, which mainly act on short-, middle-, and long-chain alkanes, respectively (58, 78). In some cases, these enzymes coexist in the same microorganism and cooperate to contribute a broad range of substrate chain lengths. In the genome of SH22a, no genes for MMOs or MMO-like enzymes (e.g., propane monooxygenase and butane monooxygenase) were detected, indicating that SH22a may not metabolize gaseous alkanes. The AlkB alkane hydroxylase system is a three-component complex composed of a hydroxylase (AlkB) and two electron carriers, namely, rubredoxin and rubredoxin reductase (79, 80). The exploration of the genome revealed that SH22a contains four putative AlkB-encoding genes (NONO_c08910, NONO_c46180, NONO_c63170, and NONO_c63220), located at three chromosomal loci. Two of them (NONO_c46180 and NONO_c63220) are found in possible operons encoding rubredoxin reductases (NONO_c46210) and/or rubredoxins (NONO_c46190, NONO_c46200, NONO_c63200, and NONO_c63210). Although these AlkBs share high amino acid sequence similarities (>70%), their substrate specificity may be heterogeneous. Whereas some catalyze the oxidation of middle-chain-length or long-chain *n*-alkanes (81), the others may also function on branched alkanes (82). In recent studies, CYP450 members were shown to represent another group of middle-chain alkane hydroxylases in both Gram-negative and Gram-positive bacteria (83). The *N. nova* SH22a genome encodes 48 CYP450 proteins (PF00067), some of which exhibit similarities to already characterized alkane hydroxylases. These include, *inter alia*, the CYP450 encoded by NONO_c15420. The corresponding protein has 33% amino acid sequence identity to CYP153A1 of *Acinetobacter* sp. EB104 and 31% amino acid sequence identity to CYP150A6 of *Mycobacterium* sp. HXN-1500; these enzymes catalyze the initial step in alkane oxidation (83). Deduced proteins showing similarity to LadA (NONO_c02520) (84, 85) or Alma (NONO_c13310, NONO_c18230, NONO_c36810, NONO_c40500, and NONO_c51810) (86, 87), which were characterized as catalyzing the terminal oxidation of long- and/or very-long-chain alkanes (up to at least C₃₆) to the corresponding primary alcohols, were also found to be encoded in the genome of SH22a, indicating that this strain may metabolize very-long-chain alkanes as well.

Secondary metabolites. PKSs, nonribosomal peptide synthetases (NRPSs), or their hybrid complexes (PKS-NRPSs) are large multimodular enzymes synthesizing a fascinating class of natural products referred to as polyketides, nonribosomal peptides, or hybrids thereof in bacteria and fungi. Many of these compounds were found to possess significant biological activities of clinical or biotechnological importance (e.g., as virulence factors, novel antibiotics, or immunosuppressive or antitumor agents) (67, 88). The biosynthesis pathways of certain metabolites have been elucidated, particularly in actinobacterial species (89–92). As with many other actinobacteria, strains of *Nocardia* species exhibit potent capabilities in the synthesis of secondary metabolites (16). In the present study, the genetic basis for the synthesis of these metabolites was examined in *N. nova*. The SH22a genome possesses, in total, 22 PKS- or NRPS-related gene clusters, among which 6 clusters contain type I PKS (PKS-I) genes, 1 cluster contains type II PKS (PKS-II) genes, 11 clusters contain NRPS genes, and 4 clusters contain PKS-NRPS genes (see the supplemental material). The genus *Streptomyces* is known to possess extensive sec-

ondary metabolic capabilities for producing novel bioactive compounds of clinical and industrial importance. The larger number of PKS and NRPS gene clusters in *N. nova* than in some *Streptomyces* species, such as *Streptomyces turgidiscabies*, *Streptomyces scabiei*, *S. coelicolor*, *Streptomyces griseus* subsp. *griseus*, and *Streptomyces avermitilis* (93), was therefore somewhat unexpected. This result provides clues that the genus *Nocardia*, which was previously known for its pathogenic and potent catabolic properties (16), could also be a valuable source for the production of useful secondary metabolism products.

Research concerning the biosynthesis pathways of polyketides and nonribosomal peptides in the genus *Nocardia* has been quite limited until now. By comparing the PKS and NRPS gene clusters of SH22a to the functionally elucidated polyketide and nonribosomal peptide biosynthesis pathways, products of most of these clusters cannot be predicted due to the relatively low amino acid sequence similarities and the distinct organization pattern of genes. However, three gene clusters are the exceptions. The gene cluster NONO_c01500 to -c01520 exhibits very high similarity to the *fadD32-pks13-accD4* operon of *M. tuberculosis* (see above) and is supposed to be involved in mycolic acid synthesis. NONO_c01510 encodes a PKS-I condensase catalyzing the last condensation step. Only one PKS-II gene cluster was detected in SH22a. This cluster contains the genes for a complete pathway for type II polyketide backbone synthesis and is therefore predicted to participate in type II polyketide product biosynthesis. The two β -ketoacyl synthase subunits (KS_{α} and KS_{β}) and the acyl carrier protein (ACP) of the minimal PKS-II are encoded by NONO_c10810, NONO_c10800, and NONO_c10790, respectively. Genes coding for a ketoreductase (NONO_c10780) and an aromatase (NONO_c10770), which are required for the subsequent cyclization of polyketide intermediates, are located directly downstream of the PKS-II genes. In addition, genes coding for proteins exhibiting similarities to ActIV (NONO_c10760), ActVIA (NONO_c10820), ActVI3 (NONO_c18340), ActVI4 (NONO_c57970), ActVA5 (NONO_c37860), and ActVB (NONO_c51280), which are involved in the biosynthesis of a well-studied antibiotic, actinorhodin, in *S. coelicolor* A3(2) (94, 95), were also found in the SH22a genome, indicating that this strain might synthesize an actinorhodin-like bioactive compound. The virulence-conferring siderophore antibiotics mycobactin and nocobactin NA are secondary metabolites produced by *M. tuberculosis* and *N. farcinica*, respectively (91, 92). The assembly of their identical core structure is accomplished by a large PKS-NRPS gene cluster: the *mbt* (*M. tuberculosis*) or *nbt* (*N. farcinica*) gene cluster. Interestingly, a similar gene cluster that contains nine PKS-NRPS genes (NONO_c08770 to NONO_c08850) was detected in SH22a. Although the gene organization is different from that of the *mbt* or *nbt* cluster, the high levels of similarity (46 to 71% amino acid sequence identity) exhibited by these genes and the existence of a siderophore-interacting protein gene (NONO_c08760) upstream of this cluster still strongly suggest its involvement in the synthesis of a siderophore product. This siderophore may play a role in iron acquisition from host cells in an infective event.

Conclusions. The elaboration of the complete genome of *N. nova* SH22a, the first bacterium capable of degrading GP (and also rubber), and the generation of transposon and deletion mutants contribute to a deeper understanding of the degradation of both polyisoprene isomers. The importance of the cell envelope synthesis genes *fadD32*_{SH22a} and *aftB* for the degradation of the two iso-

mers was clearly shown. Furthermore, genes that are (or may be) responsible for initial oxidative cleavage, uptake of the resulting degradation intermediates, metabolism of these products via β -oxidation, and central metabolism were detected. Several of the corresponding genes were confirmed by the generation of mutants. The previously described assumption that GP and rubber degradation pathways are quite similar and share common steps (15) has been confirmed. The sequencing of the SH22a genome shows that this isolate not only represents a strain of the genus *Nocardia* possessing manifold potential catabolic capabilities but also may be an interesting candidate for the synthesis of novel bioactive compounds.

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