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ORIGINAL PAPER

Complete genome sequencing and comparative CAZyme analysis of *Rhodococcus* sp. PAMC28705 and PAMC28707 provide insight into their biotechnological and phytopathogenic potential

Nisha Ghimire[1] · So-Ra Han[1] · Byeollee Kim[1] · Sang-Hee Jung[2] · Hyun Park[3] · Jun Hyuck Lee[4,5] · Tae-Jin Oh[1,6,7]

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Abstract

Study of carbohydrate-active enzymes (CAZymes) can reveal information about the lifestyle and behavior of an organism. *Rhodococcus* species is well known for xenobiotic metabolism; however, their carbohydrate utilization ability has been less discussed till date. This study aimed to present the CAZyme analysis of two *Rhodococcus* strains, PAMC28705 and PAMC28707, isolated from lichens in Antarctica, and compare them with other *Rhodococcus*, *Mycobacterium*, and *Corynebacterium* strains. Genome-wide computational analysis was performed using various tools. Results showed similarities in CAZymes across all the studied genera. All three genera showed potential for significant polysaccharide utilization, including starch, cellulose, and pectin referring their biotechnological potential. Keeping in mind the pathogenic strains listed across all three genera, CAZymes associated to pathogenicity were analyzed too. Cutinase enzyme, which has been associated with phytopathogenicity, was abundant in all the studied organisms. CAZyme gene cluster of *Rhodococcus* sp. PAMC28705 and *Rhodococcus* sp. PAMC28707 showed the insertion of cutinase in the cluster, further supporting their possible phytopathogenic properties.

Keywords CAZyme · *Rhodococcus* · Polysaccharide · Biotechnological potential · Cutinase · Phytopathogenic property

Introduction

Carbohydrate-active enzymes (CAZymes) include enzymes involved in the degradation, modification, and formation of glycosidic linkages (Garron and Henrissat 2019). They have been classified, based on amino acid similarities, into six

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✉ Jun Hyuck Lee
junhyucklee@kopri.re.kr

✉ Tae-Jin Oh
tjoh3782@sunmoon.ac.kr

Nisha Ghimire
nysaghimire8@gmail.com

So-Ra Han
553sora@naver.com

Sang-Hee Jung
yredgirl@naver.com

Hyun Park
hpark@korea.ac.kr

[1] Department of Life Science and Biochemical Engineering, Graduate School, Sun Moon University, Asan 31460, Korea

[2] Department of Dental Hygiene, Gangneung Yeongdong University, Gangneung, Korea

[3] Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea

[4] Unit of Research for Practical Application, Korea Polar Research Institute, Incheon 21990, Korea

[5] Department of Polar Sciences, University of Science and Technology, Incheon 21990, Korea

[6] Genome-Based BioIT Convergence Institute, Asan 31460, Korea

[7] Department of Pharmaceutical Engineering and Biotechnology, Sun Moon University, Asan 31460, Korea

classes, namely glycosyl hydrolase (GH), glycosyl transferase (GT), polysaccharide lyase (PL), carbohydrate esterase (CE), auxiliary activities (AA), and carbohydrate binding module (CBM), in the CAZy database (http://www.cazy.org/). CAZymes consist of both degradative and glycosidic bond-forming enzymes. While GH, CE, PL, and AA are degradative enzymes, GT is involved in glycosylation and CBM helps in substrate binding. Degradative CAZymes are largely distributed in the CAZy database, with 167 GH, 40 PL, 17 CE, and 16 AA, till date. This study focused on the degradative CAZymes, mainly GH and CE, which were found to be abundantly distributed in the two isolated *Rhodococcus* strains, *Rhodococcus* sp. PAMC28705 and *Rhodococcus* sp. PAMC28707.

CAZymes have several biotechnological and industrial potential. They play a major role in polysaccharide degradation with biofuel production. Plant biomass is a potent source of bioenergy and CAZymes deconstruct this biomass for bioenergy production (Brunecky et al. 2018). Several recalcitrant polysaccharides in biomass like cellulose, hemicellulose, and pectin degradation has been a major challenge and necessity of this century. They are utilized for the production of bioethanol along with textile, brewery, food, and paper industries (Jacob et al. 2008; Pinard et al. 2015; Ghimire et al. 2020). Also, starch, one of the major polysaccharide in plants has importance as nutrition in food additives and as a feedstock in bioethanol production (Nigam and Singh 1995; Zeeman et al. 2010). Overall, CAZymes have proven to be a major consideration for biotechnological importance.

CAZymes depict the lifestyle and behavior of organisms. Carbohydrates are utilized by all kinds of species for different purposes, such as energy, multiple inter- and intracellular signal transduction, and host–pathogen interactions (Garron and Henrissat 2019). Therefore, understanding the carbohydrate utilization ability of an organism is important to uncover their mysteries and potentials. Several studies on CAZymes have shown their involvement as a virulence factor in fungi and bacteria, inferring the pathogenic behavior of the organism (Lyu et al. 2015; Boncan et al. 2018; Ma et al. 2019). GH and CE are well-known plant cell wall degrading enzyme families in both bacteria and fungi (Ospina-Giraldo et al. 2010; Lyu et al. 2015). These enzymes act as a medium for cell wall degradation, leading to infection. Enzymes such as cutinase, cellulases, hemicellulases, and pectinases are known to degrade plant cell walls (Zhao et al. 2013). Among these, cutinases have been widely studied for their phytopathogenic properties.

Rhodococcus is a Gram-positive, aerobic, and non-spore-forming bacterium with usually high G + C content (Van Der Geize and Dijkhuizen 2004). Several strains of *Rhodococcus* have been isolated from sources such as soil, seawater, sputum, fresh water, oil-contaminated regions, and leaves, as well as from extreme environments. This genus includes both pathogenic and nonpathogenic strains. The well-known pathogenic strains of this genus are *Rhodococcus fascians* and *Rhodococcus equi*, of which the former is well known as a plant pathogen and the latter is an animal pathogen. *Rhodococcus* is renowned for hydrocarbon and xenobiotic metabolism, along with the production of various aromatic compounds. However, very few studies, till date, have focused on carbohydrate metabolism of this organism. This study aimed to explore the CAZymes of two *Rhodococcus* sp. PAMC28705 and *Rhodococcus* sp. PAMC28707, obtained from lichens in Antarctica, and compare them with other *Rhodococcus*, *Mycobacterium*, and *Corynebacterium* species to understand their biotechnological potent and pathogenic behavior with respect to CAZymes.

Materials and methods

Isolation and genomic DNA extraction of *Rhodococcus* sp. PAMC28705 and *Rhodococcus* sp. PAMC28707

We isolated two strains from the Antarctic lichens and analyzed the species by comparing their 16S rRNA sequences. To isolate bacteria from Antarctic lichens, we cut the segment of lichen thallus into smaller pieces and used 0.85% NaCl solution for washing. After washing, the lichen segment was mixed with sterilized 0.85% NaCl solution (2.0 mL). Then, the mixed lichen solution was spread on R2A medium and incubated at 15 °C for 15 days. Pure isolates were obtained, and the pure culture of bacteria was preserved at − 70 °C in 20% glycerol. The 16S rRNA gene was amplified from a single colony with two universal primers: 27F (5′- AGA GTT TGA TCM TGG CTC AG -3′) and 1492R (5′- GGT TAC CTT GTT ACG ACT T -3′). The sequence of 16S rRNA gene was matched and compared with that in species strains available in the EzBioCloud database (Yoon et al. 2017). We deposited the two strains and obtained the Polar and Alpine Microbial Collection (PAMC) numbers from to the Korea Polar Research Institute (KOPRI). Further, the whole genome was submitted to genbank with accession number.

Genome sequencing, assembly, and annotation

Genomic DNA was extracted from the harvested cells of *Rhodococcus* sp. PAMC28705 and PAMC28707 using a QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA, USA), and their quantity and purity were determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Genome sequencing was performed using PacBio RS II single-molecule real-time (SMRT)

sequencing technology (Pacific Biosciences, Menlo Park, CA, USA). SMRTbell library inserts (20 kb) were sequenced using SMRT cells. Each strain of raw sequence data was generated and assembled de novo using the hierarchical genome-assembly process (HGAP) protocol (Chin et al. 2013) and RS HGAP4 Assembly in SMRT analysis software (ver. 2.3; Pacific Biosciences [https://github.com/Pacific Biosciences/SMRT-Analysis]).

Phylogenetic placement and ANI analysis

A phylogenetic tree was constructed for *Rhodococcus* sp. PAMC28705 and *Rhodococcus* sp. PAMC28707, using the neighbor-joining tree method in MEGAX software with bootstrap value of 1000 replicates for reliability. The 16S rRNA sequence was obtained from the NCBI genome database for selected genomes of *Rhodococcus, Mycobacterium*, and *Corynebacterium* species. Further, we wanted to analyze the average nucleotide identity (ANI) of the selected microorganism with respect to *Rhodococcus* sp. PAMC28705 and PAMC28707. It was performed using the ANiM tool, based on MUMmer within the JSpeciesWS web service. This method is based on in-silico analysis for calculating the extent of nucleotide identity across genomes.

Comparative analysis

For comparative analysis, only the characterized species of the *Rhodococcus* genus, having complete genome, were selected. All the selected genomes are submitted to GeneBank, under the accession numbers as follows: *R. aetherivorans* IcdP1 (CP011341.1), *R. biphenylivorans* TG9 (CP022208.1), *R. coprophilus* NCTC10994 (LS483468.1), *R. erythropolis* CCM2595 (CP003762.1), *R. fascians* D188 (CP015237.1), *R. hoagii* DSSKP-R-001 (CP027793.1), *R. jostii* RHA1 (CP000431.1), *R. opacus* B4 (AP011115.1), *R. pyridinivorans* GF3 (CP022915.1), *R. qingshengii* RL1 (CP042917.1), *R. rhodochrous* ATCC-BAA870 (CP032675.1), and *R. ruber* P14 (CP024315.1).

Besides *Rhodococcus*, we also selected *Mycobacterium* and *Corynebacterium* from the same phylum for comparative study. They were submitted to the GenBank with accession numbers: *M. canettii* CIPT140010059 (HE572590.1), *M. intracellulare* ATCC13950 (CP003322.1), *M. tuberculosis* H37Rv (CP003248.2), *C. diphtheriae* NCTC11397 (LN831026.1), *C. glutamicum* ATCC13032 (CP025533.1), and *C. ulcerans* NCTC7910 (LT906443.1). All these genomes were obtained in FASTA format from the NCBI genome database for further analysis.

CAZyme annotation

To analyze the CAZyme-related genes in microorganisms, we subjected all the selected genomes to the dbCAN2 Meta server (http://cys.bios.niu.edu/dbCAN2). The genomes were annotated using DIAMOND, HMMER, and Hotpep, which were integrated within the dbCAN2 Meta server against CAZy, dbCAN, and PPR databases. Furthermore, the CAZyme annotated genes were subjected to NCBI BLASTp against the non-redundant database to determine their putative function.

CAZyme gene cluster analysis

The dbCAN2 Meta server includes CGC finder for finding the cluster of CAZymes that contain at least one or more CAZymes, transcription factors (TF), and transporters (TC) (Zhang et al. 2018). To include the CGC cluster result, the CGC finder tool was selected while uploading the genome in the dbCAN2 Meta server. The CAZyme annotation method was described above while TF search was conducted against a database of transcriptional regulation in *Bacillus subtilis* (DBTBS), RegulonDB, and collectTF databases, and TC was searched against the transporter classification database (TCDB), all integrated in the dbCAN2 Meta server (Huang et al. 2018).

Results

General features of *Rhodococcus* sp. PAMC28705 and *Rhodococcus* sp. PAMC28707

PAMC28705 and PAMC28707, isolated from lichens in Antarctica, showed almost similar patterns in their genome analysis. We have submitted both genomes to Genbank with accession number CP039254.1 and CP039253.1 for PAMC28705 and PAMC28707 respectively. Both species had a genome size of 4.72736 Mb, although the number of proteins varied slightly (Table 1). The genome information of all the studied strains is summarized in Table S1.

Table 1 Genomic features of *Rhodococcus* species

Criteria	*Rhodococcus* sp. PAMC28705	*Rhodococcus* sp. PAMC28707
Genome size (Mb)	4.72736	4.72736
Protein count	4117	4122
GC content (%)	62.1	62.1
Contigs	1	1
rRNA genes	12	12
tRNA genes	46	46

ANI analysis showed 99.99% similarity across the genomes (Table 2). In phylogenetic analysis, the species belonged to a similar clade as *R. fascians* D188, *R. qingshengii* RL1, *R. erythropolis* CCM2595, and *R. hoagii* DSSKP-R-001 (Fig. 1). In ANI analysis, both PAMC28705 and PAMC28707 showed higher similarity with *Corynebacterium* than with *Rhodococcus*; the highest similarity was with *C. diphtheriae* NCTC11397. Among the *Rhodococcus* species, these two strains showed maximum similarity with *R. fascians* D188 (84.6% for PAMC28705 and 84.78% for PAMC28707). However, there was no significant difference in the percentage similarity with other strains.

CAZyme analysis

The *Rhodococcus* strains PAMC28705 and PAMC28707, subjected to the dbCAN2 Meta server, showed total CAZyme percentage of 3.8% with 156 and 157 CAZymes, respectively. They showed the highest number of GT families (which was 61), followed by 52 and 53 GH families for PAMC28705 and PAMC28707, respectively, 30 CEs, 7 AAs, and 7 CBMs for both species. However, we could not find any gene related to PL in case of both. The putative degradative CAZyme functions and their respective substrates in PAMC28705 and PAMC28707 are listed in Table S2, Fig. 2. While comparing the CAZyme genes with those of other *Rhodococcus*, *Mycobacterium*, and *Corynebacterium* strains, the maximum CAZyme percentage was observed in *R. erythropolis* CCM2595 and the minimum was in *R. rhodochrous* P14 (Table S3); all of them had GH, GT, CE, AA, and CBM, and PL was absent in all (Fig. 3).

GH is the class of CAZyme that is responsible for hydrolytic cleavage of the glyosidic bond between carbohydrates or carbohydrates with a non-carbohydrate moiety (Chernysheva et al. 2019). There were 32 GH families distributed across *Rhodococcus* strains while 20 were distributed in *Mycobacterium* and 18 in *Corynebacterium*, as shown in Table S4. We categorized these GH families into starch-, glycogen-, peptidoglycan-, cellulose-, and pectin-degrading genes based on their annotation in Tables 3 and 4. PAMC28705 and PAMC28707 had genes related to all these categories. Starch- and glycogen-degrading genes were distributed across all strains of the studied genera. Cellulose-degrading enzymes were found in all three genera, although they were not distributed across all strains. All *Mycobacterium* strains contained cellulases, whereas some *Rhodococcus* and *Corynebacterium* lacked it. On the contrary, most of the pectin-degrading enzymes were distributed only in all *Rhodococcus* strains, except for arabinofuranosidase, which was only detected in PAMC28705, PAMC28707, and D188. Among the peptidoglycan-degrading enzymes, lytic transglycosylase was present in all while β-hexosaminidase/muramidase was absent in *Corynebacterium*.

GT are enzymes involved in the formation of glycosidic bonds, utilizing the activated sugar donor and transferring it to the acceptor molecule, thus forming oligosaccharides, polysaccharides, and glycoconjugates (Coutinho et al. 2003; Breton et al. 2006; Lairson et al. 2008). We found a total of 21 GT families in *Rhodococcus*, 17 in *Mycobacterium*, and 18 in *Corynebacterium*. In *Rhodococcus* strains, GT9, GT19, GT26, and GT94 are unique. In contrast, GT47 and GT76 are unique in *Corynebacterium* while no unique GT family has yet been identified in *Mycobacterium*. All of them had GT1, GT2, GT4, GT5, GT13, GT20, GT28, GT35, GT39, GT51, GT53, GT81, GT83, GT85, GT87, and GT89, as shown in Table S5. GT was found in high numbers across all the species.

CE is involved in the hydrolysis of ester bonds, to remove ester decorations from carbohydrate moieties (Nakamura et al. 2017). They generally catalyze reactions such as *O*- or

Table 2 ANI value (%) of *Rhodococcus* sp. PAMC28705 and *Rhodococcus* sp. PAMC28707 using ANiM tool

Species	PAMC28705	PAMC28707
PAMC28705		99.99
PAMC28707	99.99	
IcdP1	83.52	83.56
TG9	83.68	83.42
NCTC10994	84.17	84.07
CCM2595	84.58	84.40
D188	84.60	84.78
DSSKP-R-001	83.86	83.78
RHA1	83.91	84.13
B4	84.21	84.02
GF3	83.77	83.92
RL1	84.44	84.35
ATCCBAA870	83.64	83.55
P14	83.69	83.69
CIPT140010059	83.42	83.42
ATCC13950	83.22	83.22
H37Rv	83.40	83.40
NCTC11397	85.69	84.77
ATCC13032	85.13	85.13
NCTC7910	85.46	84.44

PAMC28705 = *Rhodococcus* sp., PAMC28707 = *Rhodococcus* sp., IcdP1 = *Rhodococcus aetherivorans*, TG9 = *Rhodococcus biphenylivorans*, NCTC10994 = *Rhodococcus coprophilus*, CCM2595 = *Rhodococcus erythropolis*, D188 = *Rhodococcus fascians*, DSSKP-R-001 = *Rhodococcus hoagii*, RHA1 = *Rhodococcus jostii*, B4 = *Rhodococcus opacus*, GF3 = *Rhodococcus pyridinivorans*, RL1 = *Rhodococcus qingshengii*, ATCCBAA870 = *Rhodococcus rhodochrous*, P14 = *Rhodococcus ruber*, CIPT140010059 = *Mycobacterium canettii,* ATCC13950 = *Mycobacterium intracellulare*, H37Rv = *Mycobacterium tuberculosis*, NCTC11397 = *Corynebacterium diphtheria*, ATCC13032 = *Corynebacterium glutamicum*, and NCTC7910 = *Corynebacterium ulcerans*

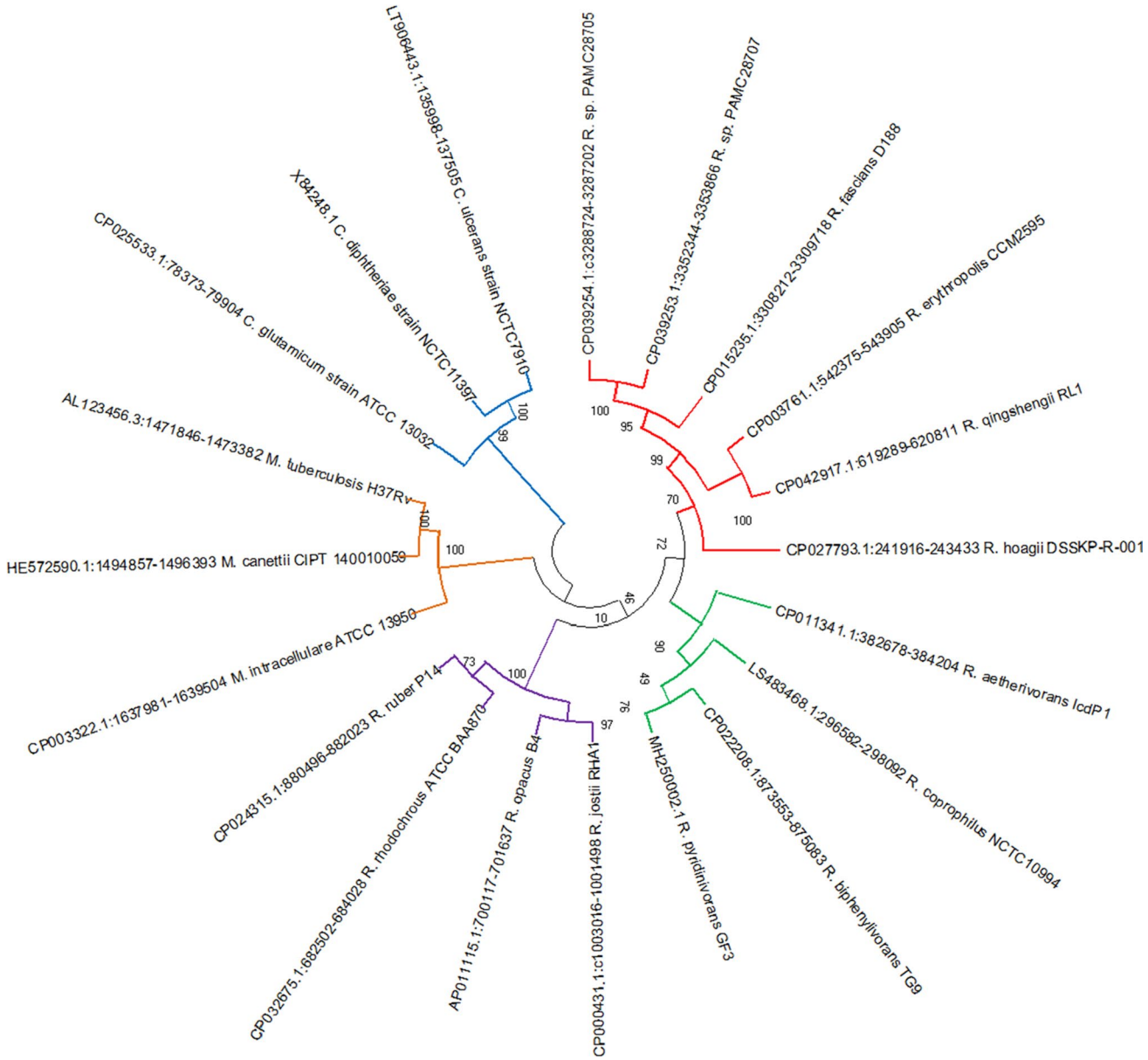

Fig. 1 Phylogenetic placement of *Rhodococcus* sp. PAMC28705 and *Rhodococcus* sp. PAMC28707 with other species of *Rhodococcus*, *Mycobacterium*, and *Corynebacterium*. The tree was developed using Mega X with bootstrap value of 1000 replicates for confidentiality

N-deacylation, thus removing the ester bonds (Cantarel et al. 2008; Park et al. 2018). *Rhodococcus* strains had ten different CE families while there were seven in both *Mycobacterium* and *Corynebacterium* (Table S6). We classified these esterases into cutinase, *N*-acetylglucosamine-6-phosphate de-*N*-acetylase, *N*-acetyl-D-glucosaminylphosphatidylinositol de-*N*-acetylase, *S*-formylglutathione hydrolase, and mycothiol *S*-conjugate amidase, based on their annotation results (Table 5). Among them, cutinase and *S*-formylglutathione hydrolase were present in all species, *S*-formylglutathione hydrolase being the most abundant, followed by cutinase.

AA are enzymes that break down glycosidic linkages through oxidation reaction (Chernysheva et al. 2019). In this study, we found a total of eight families of AA in *Rhodococcus*, six in *Mycobacterium*, and four kinds in *Corynebacterium*. *Rhodococcus* species had the AA families, namely AA1, AA2, AA3, AA4, AA5, AA6, AA7, and AA8. *Mycobacterium* did not contain AA4 and AA5, whereas *Corynebacterium* lacked AA2, AA5, and AA6 (Table S4). The most abundant AA families were AA3 and AA7, found in all the strains. With respect to the AA3 family, all the annotated *Rhodococcus*, *Mycobacterium,* and *Corynebacterium* strains showed choline dehydrogenase

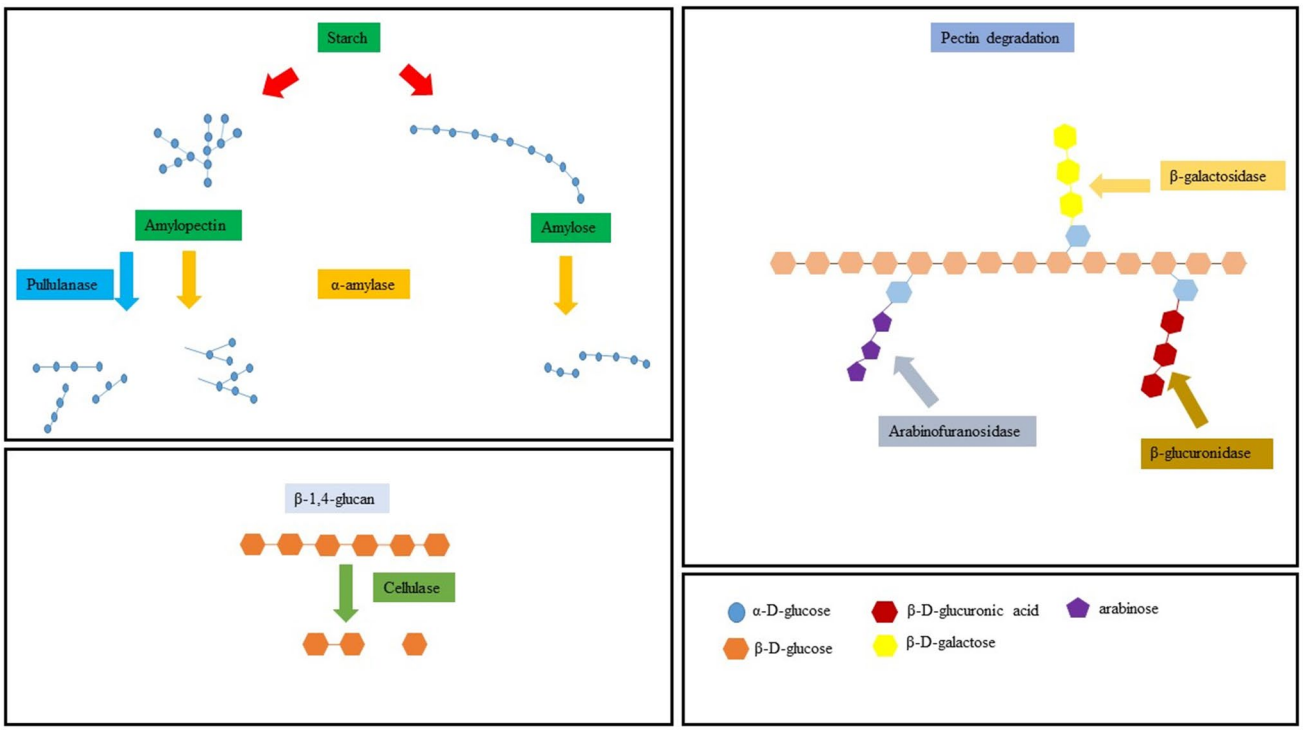

Fig. 2 Major polysaccharide utilization mechanism by *Rhodococcus sp*. PAMC28705 and *Rhodococcus sp*. PAMC28707

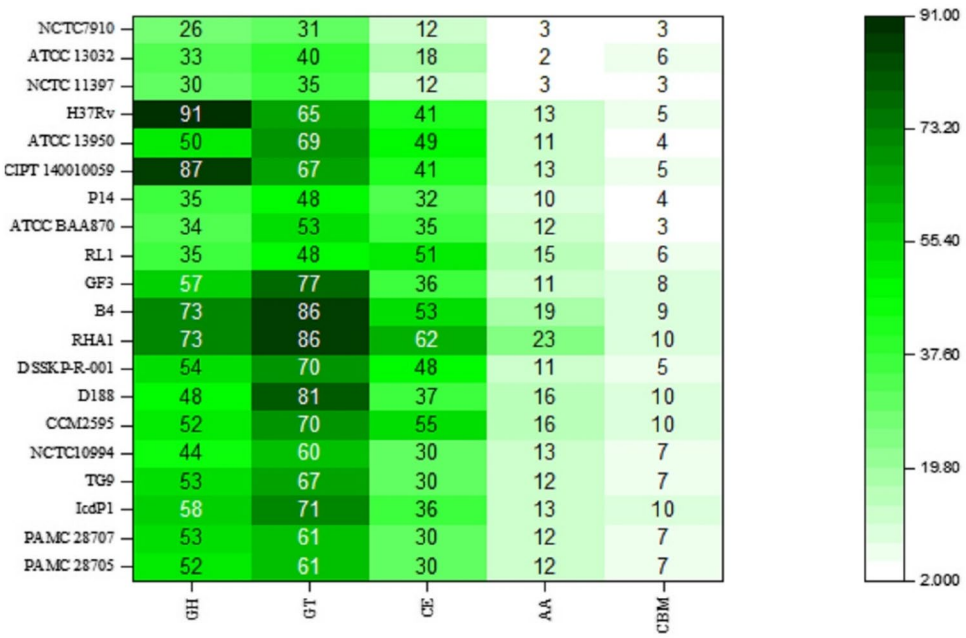

Fig. 3 Heatmap showing the CAZyme classification Glycosyl hydrolase (GH), Glycosyl transferase (GT), Carbohydrate esterase (CE), Auxiliary activities (AA), and Carbohydrate binding module (CBM) across all of *Rhodococcus*, *Mycobacterium*, and *Corynebacterium* species compared. The color bar represents decreasing order of the number with intensity of color (color figure online)

activity. Some AA3 showed a combination with AA8, both having the same function as choline dehydrogenase, during annotation. Similarly, the AA7 family in our annotated genes of three different genera showed functions like genes encoding FAD/FMN-dependent dehydrogenases.

CBM is a non-catalytic polysaccharide-binding module that plays a vital role in determining the accessibility of the substrate to the enzyme (Boraston et al. 2004). CBMs were found to be associated with GH and CE families. *Rhodococcus* contained seven families of CBM, including CBM2,

Table 3 Different glycosyl hydrolase (GH) families and the amount of genes involved for starch and glycogen degradation

Species	Starch				Glycogen	
	α-amylase/glucoamylase		pullulanase/α-1,6-glucosidase		glycogen debranching enzyme	
PAMC28705	GH13, GH57, GH77, GH15	12	GH13, GH57, GH77, GH63	5	GH13, GH77	3
PAMC28707	GH13, GH15, GH77	11	GH13, GH77	3	GH13, GH77	3
IcdP1	GH0, GH13,GH77,GH15	15	GH13, GH77	2	GH13, GH77	2
TG9	GH0, GH13,GH15, GH77	12	GH13, GH77	2	GH13, GH77	2
NCTC10994	GH0, GH13,GH15, GH77	10	GH13, GH77	2	GH13, GH77	2
CCM2595	GH13, GH15, GH77	7	GH13, GH77	2	GH13, GH63, GH77	3
D188	GH13, GH15, GH77	12	GH13, GH77	3	GH13, GH63, GH77	4
DSSKP-R-001	GH13, GH77	6	GH13, GH77	1	GH13, GH77	2
RHA1	GH13, GH15, GH77	10	GH13, GH77	2	GH13, GH63, GH77	3
B4	GH0, GH13, GH77	10	GH13, GH77	2	GH13, GH63, GH77	3
GF3	GH0, GH13, GH15, GH77	12	GH13, GH77	2	GH13, GH77	2
RL1	GH13, GH15, GH77	8	GH13, GH77	2	GH13, GH63, GH77	3
ATCCBAA870	GH0, GH13,GH15	10	GH13, GH77	2	GH13, GH63, GH77	3
P14	GH0, GH13, GH15, GH77	14	GH13, GH77	2	GH13, GH63, GH77	3
CIPT140010059	GH13, GH15, GH77	6	GH13, GH77	1	GH13, GH77	1
ATCC13950	GH0, GH13, GH15, GH77	12	GH13, GH77	2	GH13, GH77	2
H37Rv	GH13, GH15, GH77	7	GH13, GH77	1	GH13, GH77	1
NCTC11397	GH13, GH77	6	GH13, GH77	2	GH13, GH77	1
ATCC13032	GH13, GH15, GH77	7	GH13, GH77	1	GH13, GH77	1
NCTC7910	GH13, GH15	7	GH13, GH77	2	GH13, GH77	1

PAMC28705 = *Rhodococcus* sp., PAMC28707 = *Rhodococcus* sp., IcdP1 = *Rhodococcus aetherivorans*, TG9 = *Rhodococcus biphenylivorans*, NCTC10994 = *Rhodococcus coprophilus*, CCM2595 = *Rhodococcus erythropolis*, D188 = *Rhodococcus fascians*, DSSKP-R-001 = *Rhodococcus hoagii*, RHA1 = *Rhodococcus jostii*, B4 = *Rhodococcus opacus*, GF3 = *Rhodococcus pyridinivorans*, RL1 = *Rhodococcus qingshengii*, ATCCBAA870 = *Rhodococcus rhodochrous*, P14 = *Rhodococcus ruber*, CIPT140010059 = *Mycobacterium canettii,* ATCC13950 = *Mycobacterium intracellulare*, H37Rv = *Mycobacterium tuberculosis*, NCTC11397 = *Corynebacterium diphtheria*, ATCC13032 = *Corynebacterium glutamicum*, and NCTC7910 = *Corynebacterium ulcerans*

CBM13, CBM32, CBM38, CBM48, CBM50, and CBM67. *Mycobacterium* only contained CBM48 and CBM50, whereas *Corynebacterium* contained CBM5, CBM48, and CBM50, as shown in Table S4. CBM48 was associated with GH13 and GH77 while CBM50 was associated with CE1, and other CBMs were independently present in CAZyme annotation.

Cutinase and phytopathogenicity

Cutinase is an enzyme involved in the degradation of plant cuticles. It is classified into CE family 5. It belongs to the superfamily of α/β-hydrolase fold proteins with a conserved GXSXG motif and Ser-His-Asp catalytic triad (Chen et al. 2008). The plant cuticle consists of cutin, composed of hydroxyl-fatty acid polyester embedded in waxes (Lu et al. 2018). Most of the aerial parts of plants, such as flowers, leaves, fruits, and stems, are covered with cuticle (Fett et al. 1992). Cutinase has been reported as the first line of invasion for phytopathogenic organisms invading the aerial parts (Fett et al. 1992; Fan and Köller 1998; Lu et al. 2018). It has been more widely studied in fungi than in bacteria (Schäfer 1993; Wang et al. 2002); however, interest in bacterial cutinase has been growing recently owing to its multifunctional application (Xu 2020).

Upon analysis of all the *Rhodococcus*, *Mycobacterium*, and *Corynebacterium* strains, we found cutinase to be distributed throughout the organism. *Rhodococcus* strains PAMC28705 and PAMC28707 had three cutinases while a maximum of 11 cutinases was present in *M.* sp. ATCC13950, followed by 7 in *M.* sp. CIPT140010059 and *M.* sp. H37Rv each. Multiple sequence alignment of cutinase from all three studied genera, with reference to the well-known cutinase from *Fusarium solani* pisi (GenBank accession no. P00590) (Egmond and De Vlieg 2000) and *A. flavus* (GenBank accession no. EED52785.1), showed the presence of conserved residues (Fig. S1). In the phylogenetic tree developed using these cutinases, we found cutinase from *Mycobacterium* strains in a separate clade. However, the

Table 4 Different glycosyl hydrolase (GH) families and the amount of genes involved for cellulose, pectin, and peptidoglycan degradation

Species	Cellulose		Pectin						Mannan		Peptidoglycan			
	β-glucosidase/cellulase		β-galactosidase		β-glucuronidase		Arabinofuranosidase		α-1,6 mannanase		β-hexosaminidase/muramidase		Lytic transglycosylase	
PAMC28705	GH1	1	GH1, GH2	2	GH2	1	GH43	1	GH76	1	GH3	1	GH23	3
PAMC28707	GH1	1	GH1, GH2	2	GH2	1	GH43	1	GH76	1	GH3	1	GH23	4
IcdP1	0	0	GH1	1	0	0	0	0	GH76	1	GH3	1	GH23	4
TG9	0	0	GH2	1	GH2	1	0	0	GH76	1	GH3	1	GH23	8
NCTC10994	GH1	1	GH1, GH2	2	GH2	1	0	0	GH76	1	GH3	1	GH23	8
CCM2595	GH3, GH5	2	GH1, GH2	3	GH2	1	0	0	GH76	1	GH25	1	GH23	7
D188	0	0	GH2	1	GH2	1	GH43	1	GH76	1	0	0	GH23	4
DSSKP-R-001	GH3	1	GH1	2	0	0	0	0	GH76	1	GH3	1	GH23	3
RHA1	GH3	1	GH1	2	0	0	0	0	GH76	1	GH25	1	GH23	8
B4	0	0	GH1, GH2, GH42	3	GH2	1	0	0	GH76	1	GH25	1	GH23	7
GF3	GH3	1	GH2	1	GH2	1	0	0	GH76	1	GH25	1	GH23	4
RL1	GH1, GH3, GH5	4	GH1, GH2	3	GH2	1	0	0	GH76	1	GH25	1	GH23	5
ATCCBAA870	GH3	1	GH2	1	GH2	1	0	0	GH76	1	0	0	GH23	6
P14	GH1,GH3	2	GH1	1	0	0	0	0	GH76	1	0	0	GH23	3
CIPT 140010059	GH6	1	0	0	0	0	0	0	GH76	1	0	0	GH23	6
ATCC13950	GH3, GH6	2	0	0	0	0	0	0	GH76	1	0	0	GH23	5
H37Rv	GH3, GH6	3	0	0	0	0	0	0	GH76	1	0	0	GH23	7
NCTC11397	GH3	1	0	0	0	0	0	0	GH76	1	0	0	GH23	2
ATCC13032	GH1	1	GH1	1	0	0	0	0	GH76	1	GH3	1	GH23	2
NCTC7910	0	0	0	0	0	0	0	0	GH76	1	GH25	1	GH23	1

PAMC28705 = *Rhodococcus* sp., PAMC28707 = *Rhodococcus* sp., IcdP1 = *Rhodococcus aetherivorans*, TG9 = *Rhodococcus biphenylivorans*, NCTC10994 = *Rhodococcus coprophilus*, CCM2595 = *Rhodococcus erythropolis*, D188 = *Rhodococcus fascians*, DSSKP-R-001 = *Rhodococcus hoagii*, RHA1 = *Rhodococcus jostii*, B4 = *Rhodococcus opacus*, GF3 = *Rhodococcus pyridinivorans*, RL1 = *Rhodococcus qingshengii*, ATCCBAA870 = *Rhodococcus rhodochrous*, P14 = *Rhodococcus ruber*, CIPT140010059 = *Mycobacterium canettii,* ATCC13950 = *Mycobacterium intracellulare*, H37Rv = *Mycobacterium tuberculosis*, NCTC11397 = *Corynebacterium diphtheria*, ATCC13032 = *Corynebacterium glutamicum*, and NCTC7910 = *Corynebacterium ulcerans*

cutinase from *Rhodococcus* and *Corynebacterium* showed synteny with each other (Fig. S2).

We also analyzed the CAZyme gene cluster (CGC) in PAMC28705 and PAMC28707 using CGC finder to further analyze the presence of cutinase activity in the cluster. We were able to find a cluster that incorporated two types of CAZymes, cutinase from the CE5 family, and choline dehydrogenase from the AA3 and AA8 families. In addition, the cluster contained two transporters, one transcription factor, and two unknown genes in both PAMC28705 and PAMC28707, as shown in Fig. 4.

Discussion

This study presented the carbohydrate utilization ability of two *Rhodococcus* species, PAMC28705 and PAMC28707, and their comparison with other strains from *Rhodococcus*, *Mycobacterium*, and *Corynebacterium*. We selected *Mycobacterium* and *Corynebacterium* for the comparative study owing to their close resemblance to *Rhodococcus* (Bell et al. 1998; Mistry et al. 2006). Here, we wanted to further explore their similarities with respect to CAZymes. The phylogenetic tree and ANI analysis showed the closest synteny between the two isolated *Rhodococcus* strains. In addition, they were closest to *R. fascians* D188 and *C. diphtheria*. The strain *Rhodococcus fascians* D188 is a well-known plant pathogen that is known to cause plant infection by producing or modulating plant growth regulators such as auxins and cytokinins (Stamler et al. 2016). Even though the percentage similarity of PAMC28705 and PAMC28707 did not reach the ANI threshold of > 95% for species delineation, it did indicate its potency as a pathogen. Here, selection of the characterized species for comparative analysis refers to the identification of closely related species for the isolated uncharacterized *Rhodococcus*. However, the results suggested the possibility of a novel species.

CAZyme analysis showed similarities across the studied genera. Comparative analysis of the CAZyme-related genes in PAMC28705 and PAMC28707 with those of other

Table 5 Different carbohydrate esterase family and the amount of genes involved for cutin, *N*-acetylglucosamine-6-phosphate (GlcNAc-6-phosphate), *N*-acetyl-D-glucosaminylphosphatidyl inositol (GlcNAc-PI), mycothiol *S*-conjugate, and *S*-formylglutathione degradation

Species	cutin (Cutinase)		GlcNAc-6-phosphate (GlcNAc-6-phosphate *N*-deacetylase)		GlcNAc-PI (GlcNAc-PI *N*-deacetylase)		mycothiol *S*-conjugate (mycothiol S-conjugate amidase)		*S*-formylglutathione (*S*-formylglutathione hydrolase)	
PAMC28705	CE5	3	0	0	CE14	2	CE14	1	CE1	7
PAMC28707	CE5	3	0	0	CE14	3	0	0	CE1	7
IcdP1	CE5	2		0	CE14	2	CE14	1	CE1	8
TG9	CE5	2	0	0	CE14	1	CE14	1	CE1	6
NCTC10994	CE5	2	0	0	CE14	1	CE14	1	CE1	10
CCM2595	CE5	2	0	0	CE14	2	CE14	1	CE1	14
D188	CE5	3	0	0	CE14	1	CE14	1	CE1	9
DSSKP-R-001	CE5	2	0	0	CE14	2	0	0	CE1	10
RHA1	CE5	3	0	0	CE14	1	0	0	CE1	12
B4	CE5	2	0	0	CE14	2	CE14	1	CE1	12
GF3	CE5	2	0	0	CE14	1	0	0	CE1	7
RL1	CE5	2	0	0	CE14	3	0	0	CE1	14
ATCCBAA870	CE5	2	0	0	CE14	2	CE14	1	CE1	9
P14	CE5	2	0	0	CE14	3	0	0	CE1	7
CIPT140010059	CE5	7	CE9	1	0	0	CE14	1	CE1	5
ATCC13950	CE5	11	CE9	1	CE14	1	0	0	CE1	3
H37Rv	CE5	7	CE9	1	0	0	0	0	CE1	5
NCTC11397	CE5	1	CE9	1	CE14	1	CE14	1	CE1	4
ATCC13032	CE5	1	CE9	1	CE14	2	CE14	1	CE1	6
NCTC7910	CE5	1	CE9	1	CE14	1	CE14	1	CE1	3

PAMC28705 = *Rhodococcus* sp., PAMC28707 = *Rhodococcus* sp., IcdP1 = *Rhodococcus aetherivorans*, TG9 = *Rhodococcus biphenylivorans*, NCTC10994 = *Rhodococcus coprophilus*, CCM2595 = *Rhodococcus erythropolis*, D188 = *Rhodococcus fascians*, DSSKP-R-001 = *Rhodococcus hoagii*, RHA1 = *Rhodococcus jostii*, B4 = *Rhodococcus opacus*, GF3 = *Rhodococcus pyridinivorans*, RL1 = *Rhodococcus qingshengii*, ATCCBAA870 = *Rhodococcus rhodochrous*, P14 = *Rhodococcus ruber*, CIPT140010059 = *Mycobacterium canettii,* ATCC13950 = *Mycobacterium intracellulare*, H37Rv = *Mycobacterium tuberculosis*, NCTC11397 = *Corynebacterium diphtheria*, ATCC13032 = *Corynebacterium glutamicum*, and NCTC7910 = *Corynebacterium ulcerans*

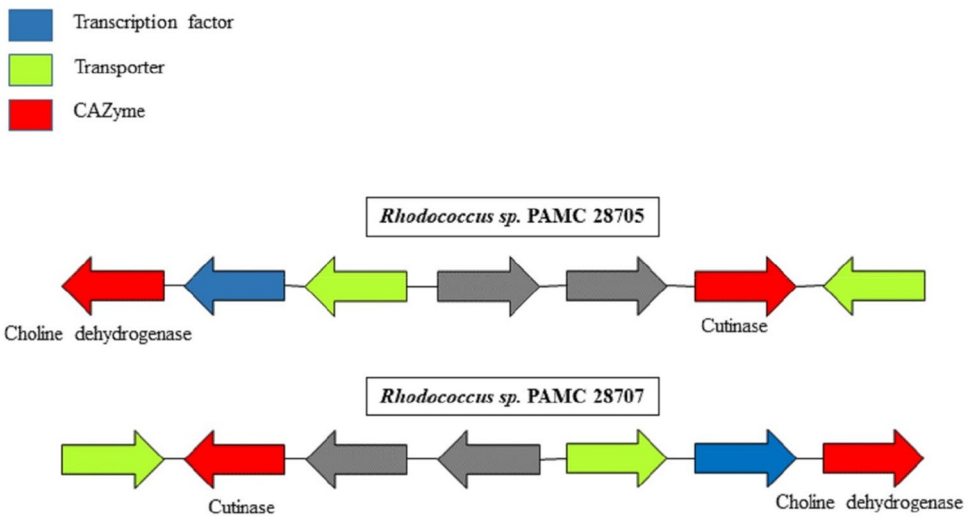

Fig. 4 CAZyme gene cluster for *Rhodococcus* sp. PAMC28705 and *Rhodococcus* sp. PAMC28707 obtained from dbCAN2 Meta server and CGC finder tool. Blue color, transcription factor; green color, transporter; and red color, CAZyme (color figure online)

strains of *Rhodococcus*, *Mycobacterium*, and *Corynebacterium* showed presence of similar degradative genes for most of the substrates such as starch, glycogen, and peptidoglycan, across the GH families observed. Likewise, cutinase, possibly associated with phytopathogenicity, was present in all the strains too. From this, we could decipher their similar polysaccharide preferences and behavior, even though they showed differences in cellulose- and pectin-degrading genes. None of the earlier studies had compared the CAZyme-related genes considering these three species together. However, one study had compared the cell wall arabinogalactans between *Rhodococcus* and *Mycobacterium* (Daffe et al. 1993) while another had compared galactan biosynthesis between *Mycobacterium* and *Corynebacterium* (Wesener et al. 2017), which are mostly related to the role of GT. However, this study mainly highlighted the degradative CAZymes.

The presence of major polysaccharides cellulose, pectin, starch, and glycogen-degrading capacity of PAMC28705 and PAMC28707 refers to the industrial application of these species. Both the strains can be used for whole cell biotransformation for complete degradation of starch with α-1, 4-glucan and α-1, 6-glucan branching with amylase and pullulanase enzymes. Likewise, cellulose can be degraded with cellulase enzyme while pectin like β-galactose, β-glucuronan, and arabinofuran can be degraded with enzymes like β-galactosidase, β-glucuronidase, and arabinofuranosidase. Similarly, *Rhodococcus opacus* PD630 was found to have starch, cellulose, and pectin-degrading activity in the previous study (Hetzler et al. 2013).

Our study was more inclined to discover CAZymes that play a role in pathogenicity too, keeping the virulence of studied genera in mind, as well as the specific role played by them. The genera *Rhodococcus*, *Mycobacterium*, and *Corynebacterium* have been reported to show pathogenic behavior (Hondalus 1997; Vereecke et al. 2002; Rogers et al. 2011; Thapa et al. 2019). Many studies have been conducted regarding the role of CAZyme in pathogenicity; however, such studies were limited to fungi (Blanco-Ulate et al. 2014; Lyu et al. 2015; Looi et al. 2017). A study of fungal CAZymes revealed the role of CAZyme in host–pathogen interactions, and most of the CAZymes have been associated with plant cell wall and fungal cell wall degradation (Lyu et al. 2015). In addition, few other studies have inferred the role of pathogenicity by bacterial CAZymes (Kolattukudy et al. 1987; Dik et al. 2017; Thapa et al. 2017), which has been highlighted in this study as well.

Cutinase play a role in phytopathogenicity. Several other enzymes, such as cellulases, pectin, and pectinases, have also shown such a role; however, most studies on these enzymes have been related to biomass degradation (Jaramillo et al. 2015; Obeng et al. 2017). Cutinase, however, has been mostly highlighted for its pathogenicity. A previous study had analyzed the cutinase activity of pathogenic and nonpathogenic fungal isolates and found the role of cutinase in pathogenicity; it was not observed, or was very low, among nonpathogenic isolates (Morid et al. 2009). Similarly, cutinase was previously listed among the plant cell wall degrading enzymes, based on CAZyme comparison, thus highlighting its significance in plant infection (Zhao et al. 2013). In another study, mutational inactivation of the virulence gene resulted in a decrease in cutinase activity, supporting the role of cutinase in virulence (Ma et al. 2019). Based on these studies, we could relate cutinase from our genome to play a role in pathogenicity.

The CGC further supported the presence of cutinase activity and its potential role in phytopathogenicity in *Rhodococcus* sp. PAMC28705 and *Rhodococcus* sp. PAMC28707. The choline dehydrogenase enzyme in the cluster led us to investigate its role. Choline is commonly found in the membrane of plants, and acts as an osmolyte for protecting plants against drought conditions (McNeil et al. 2001). It is normally produced by plants in the cytosol and serves as the polar head of phosphatidylcholine in the membrane (Summers and Weretilnyk 1993). During infection, the plant loses water, manifesting the release of choline (Scott et al. 2017). Therefore, we proposed, choline dehydrogenase enzyme might also have function in the pathogenic role of the strains. However, experimental approach is further required for confirmation.

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Compliance with ethical standards

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

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