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Unraveling the skatole biodegradation process in an enrichment consortium using integrated omics and culture-dependent strategies

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ABSTRACT

3-Methylindole (skatole) is regarded as one of the most offensive compounds in odor emission. Biodegradation is feasible for skatole removal but the functional species and genes responsible for skatole degradation remain enigmatic. In this study, an efficient aerobic skatole-degrading consortium was obtained. *Rhodococcus* and *Pseudomonas* were identified as the two major and active populations by integrated metagenomic and metatranscriptomic analyses. Bioinformatic analyses indicated that the skatole downstream degradation was mainly via the catechol pathway, and upstream degradation was likely catalyzed by the aromatic ring-hydroxylating oxygenase and flavin monooxygenase. Genome binning and gene analyses indicated that *Pseudomonas, Pseudoclavibacter*, and *Raineyella* should cooperate with *Rhodococcus* for the skatole degradation process. Moreover, a pure strain *Rhodococcus* sp. DMU1 was successfully obtained which could utilize skatole as the sole carbon source. Complete genome sequencing showed that strain DMU1 was the predominant population in the consortium. Further crude enzyme and RT-qPCR assays indicated that strain DMU1 degraded skatole through the catechol ortho-cleavage pathway. Collectively, our results suggested that synergistic degradation of skatole in the consortium should be performed by diverse bacteria with *Rhodococcus* as the primary degrader, and the degradation mainly proceeded via the catechol pathway.

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Introduction

3-Methylindole (skatole) is among the most notorious compounds contributing to odor emission in animal excrements, swine manure, activated sludges, and public latrines (Ma et al., 2021). The skatole concentration could reach as high as mg/L or mg/kg level in these environments, while its odor detection threshold is as low as 0.03 μg/m^3 in the air (Ma et al., 2021; Nagata, 2003). Skatole is generated from

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the anaerobic bacterial transformation of tryptophan and indole-3-acetic via indoleacetate decarboxylase (Liu et al., 2018). Thus, we are in intimate contact with skatole since it is continuously produced in our guts by enteric bacteria (Herter, 1908). Generally, skatole is toxic, mutagenic, and probably carcinogenic which can induce DNA fragmentation, DNA-adduct formation, and cell apoptosis (Weems et al., 2009). The transformation pathways and genes of skatole in animals and humans have been extensively investigated. P450 enzymes could convert skatole to diverse metabolites, including 3-methyloxindole, 3-hydroxy-3-methyloxindole, 3-methyleneindolenine, 3-hydroxy-3-methylindolenine, and hydroxyskatoles (Wesoly and Weiler, 2012). However, the environmental fate of skatole remains obscure.

During the last 50 years, a few aerobic bacterial strains, including *Pseudomonas aeruginosa* Gs (Yin et al., 2006), *Pseudomonas putida* LPC24 (Li et al., 2010), *Rhodopseudomonas palustris* WKU-KDNS3 (Sharma et al., 2015), *Cupriavidus* sp. strain KK10 (Fukuoka et al., 2015), *Acinetobacter* strains NTA1-2A and TAT1-6A (Tesso et al., 2019), and *Burkholderia* sp. IDO3 (Ma et al., 2020b), have been isolated with skatole-degrading capacity. Most of the reported strains are Gram-negative. The skatole degradation characteristics and potential intermediates of these strains were investigated, but few efforts have been made to illuminate the genetic biotransformation mechanisms. There are sporadic studies that attempted to identify the enzymes for skatole transformation. Fujioka and Wada (1968) proved that an indole-induced bacterium could oxidize skatole to 2-oxo-3-methyl-3hydroxyindole, while the enzymes were associated with the cellular debris and not successfully purified. Meng et al. (2013) obtained a potential skatole-degrading protease from the fermentation broth of strain *Lactobacillus brevis* 1.12, whereas no gene sequence and further investigations were available. Generally, aromatic aerobic degradation can be initiated via oxidation by oxygenases. Some common central intermediates, including catechol, protocatechuate, and gentisate, are generated, which are further metabolized by ring-cleaving dioxygenases (Fuchs et al., 2011). However, the functional enzymes catalyzing skatole degradation in bacteria are still a black box to date.

In environmental bioprocesses, the degradation of organic pollutants primarily results from the combined effects of microbial populations rather than a single species (Hu et al., 2021). Few species can initiate degradation and mineralize xenobiotics, and some microorganisms may be involved in the partial metabolism process by using intermediates (Qi et al., 2021; Hu et al., 2021). As for skatole degradation, there is only one report from our group describing the microbial community information based on amplicon sequencing to our knowledge, and the functional bacteria in the community remains unknown (Ma et al., 2020a). It is challenging to reveal the key species and microbial interactions between community members in the degradation process. Metagenomics is widely used recently which will provide entire gene information of a given sample. Meanwhile, metatranscriptomics which reflects the gene expression at the mRNA level will shed light on the molecular response. Integrated metagenomic and metatranscriptomic analyses have been recently applied in the degradation studies of several pollutants, including aromatic-aliphatic copolyester plastic (Meyer-Cifuentes et al., 2020), bisphenol A (Yu et al., 2019), bromoxynil and acetamiprid (Achermann et al., 2019), and crude oil (Tremblay et al., 2019), successfully revealing the potential degraders and genes.

In this study, an efficient skatole-degrading microbial consortium was enriched and characterized. Metagenomic and metatranscriptomic sequencing were conducted which unraveled the active populations and possible functional genes involved in skatole degradation. In addition to the culture-independent techniques, traditional pure culture studies were also performed to validate the prediction by omics analyses.

1. Materials and methods

1.1. *Chemical and medium*

Mineral salt medium (MSM) contains $(NH_4)_2SO_4$ 134 mg, KH_2PO_4 141 mg, K_2HPO_4 287 mg, $MnSO_4·H_2O$ 2.68 mg, $MgSO_4·7H_2O$ 21.4 mg, $FeCl_3·6H_2O$ 0.134 mg, and $CaCl_2$ 3.8 mg per liter of water. LB medium contains yeast extract 5.0 g, peptone 10.0 g, and NaCl 10.0 g per liter of water. Skatole was purchased from Aladdin (China) and dissolved in acetone as the stock solution. All chemicals and solvents were of analytical reagent grade or above.

1.2. *Enrichment and characterization of the skatole degradation consortium*

The workflow of this study was provided in **Appendix A Fig. S1**. The initial sludge sample was taken from an aerobic activated sludge reactor, which was fed with skatole and glucose in our lab (Ma et al., 2020a). The sludge sample was centrifuged and transferred to a flask fed with skatole as the sole carbon source. The culture medium was replaced every three days with increasing skatole concentrations from 50 to 200 mg/L. After a four-month operation under 30°C, 150 r/min conditions, a stable skatole-degrading consortium was obtained. To explore the skatole degradation ability of the consortium, skatole concentration was determined at certain intervals. Furthermore, the consortium (inoculum size 2%) was inoculated into fresh MSM with certain concentrations of skatole, and the skatole degradation curves were measured. Skatole degradation under different temperature (20°C, 25°C, 30°C, 35°C, 40°C) and pH (4.0, 5.0, 6.0, 7.0, 9.0, 10.0) conditions were also investigated. The skatole concentration was quantified by ultra-performance liquid chromatography with TUV detector at 280 nm (UPLC, Waters ACQUITY, USA). The sample was treated with an equal volume of methanol and filtered by a 0.22 µm filter before detection. The detection condition was 0-4 min 50:50 (V/V) water/methanol, flow rate 0.4 mL/min, and column temperature 40°C.

1.3. *Metagenomic sequencing and analyses*

One sample on the final day of operation was collected for metagenomic sequencing (designated as MS) on Illumina HiSeq 2500 platform at Guangdong Magigene Biotechnology Co., Ltd. (China). The raw sequences were filtered by Trimmomatic (LEADING:3 TRAILING:3 SLIDINGWINDOW:5:20

MINLEN:50) (Bolger et al., 2014), clean reads were *de novo* assembled by MEGAHIT (k-min 35, k-max 95, k-step 20) (Li et al., 2015), and scaftigs (without chimeras) with length >500 bp were selected for the following analyses. The opening reading frames (ORFs) were predicted by Prodigal and clustered by Linclust to obtain gene catalogues (Hyatt et al., 2010; Steinegger and Söding, 2018). The abundance of each gene (ppm, one read in one million reads) was calculated with the help of BBmap software (Bushnell, 2014). To obtain the genomes of the potential skatole-degrading strains, scaftigs were utilized to perform binning by taking tetranucleotide frequency and coverage values into consideration using the MetaBAT2 v2.11.2 (Kang et al., 2019). The completeness and contamination of bins were estimated using CheckM v1.0.7 (Parks et al., 2015). Bins with completeness up to 90% and contamination lower than 10% were kept and the taxonomic annotation was completed based on the GBTK database. Gene prediction for the bins was conducted using Prodigal v 2.6.3 with the "-p meta" option. For functional annotation, the nonredundant gene catalogues were aligned against the NCBI-NR database (e-value≤0.0001), non-supervised Orthologous Groups (eggNOG) database (e-value≤0.001), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (e-value≤0.001).

1.4. Metatranscriptomic sequencing and gene expression quantitation

The sample for metagenomic sequencing was simultaneously sent for metatranscriptomic sequencing (designated as TS) at Guangdong Magigene Biotechnology Co., Ltd. (China). In addition, the microbial consortium was centrifuged and washed with the fresh medium after the skatole was completely removed. Then it was transferred to the fresh medium with glucose as the carbon source in the absence of skatole. This sample (designated as TG) was also collected for metatranscriptomic sequencing (**Appendix A Fig. S1**). The quality filter of metatranscriptomic reads was consistent with the metagenome by Trimmomatic (Bolger et al., 2014). Clean reads were mapped to the ORFs identified by metagenome for both assembly and binning methods by BBmap, and the expression levels of each gene were calculated.

1.5. Isolation of strain Rhodococcus sp. DMU1

The traditional spread plate technique was adopted to screen skatole-degrading strains from the consortium using MSM with skatole as the sole carbon source. One red-colored colony was identified by amplifying, sequencing, and analyzing the 16S rRNA gene. The phylogenetic tree of the strain DMU1 was constructed by MEGA7 using the Neighbor-Joining method (bootstrap test 1000 replicates) (Kumar et al., 2016). The skatole degradation assays of the strain DMU1 were carried out in MSM in triplicate. The growth of strain was monitored by the UV-spectrophotometer (UV-9000S, China) at 600 nm wavelength, and the residual skatole in the medium was determined by UPLC. The Grain staining was performed as described elsewhere (Beveridge, 2001). The morphology of bacterial cells was observed using a scanning electron microscope as previously reported (Qu et al., 2015).

1.6. Genome sequencing of the strain DMU1

Total DNA of the strain DMU1 was extracted using FastPure Bacteria DNA Isolation Mini Kit (Vazyme, China), which was further treated and sequenced at Guangdong Magigene Biotechnology Co., Ltd. (China) on Illumina and PacBio Sequel platforms. The low-quality data were treated and assembled with the help of SMRT Link v5.0.1 and Unicycler (Chin et al., 2013; Wick et al., 2017). The genome sequence was submitted to the GenBank database and annotated by NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The average nucleotide identity (ANI) analysis was performed using JSpeciesWS (Richter et al., 2016), and genome-based identification was also conducted via Type (Strain) Genome Server (Meier-Kolthoff and Göker, 2019).

1.7. Crude enzyme activity assay

Cells of the strain DMU1 were cultured in LB medium (containing 50 mg/L skatole) for 24 hr and harvested by centrifugation (6000 r/min, 5 min, 4°C). The cell pellets were washed, resuspended, and sonicated in phosphate buffer (50 mmol/L, pH 7.4). The sonication mixture was further centrifugated (10000 r/min, 20 min, 4°C), and the clear supernatant was used as the crude enzyme extraction. The enzyme activities of catechol 1,2-dioxygenase (C12O), catechol 2,3-dioxygenase (C23O), protocatechuate 2,3-dioxygenases (P23O), protocatechuate 3,4-dioxygenases (P34O), protocatechuate 4,5-dioxygenases (P45O), and gentisate 1,2-dioxygenase (G12O) were determined as previously reported (Shen et al., 2004; Zhou et al., 2001). Briefly, the enzyme reaction was performed in a 2 mL system in the tube with 100 mmol/L catechol (50 mmol/L phosphate buffer with 20 mmol/L Na_2EDTA, pH 7.4), protocatechuate (50 mmol/L Tris-HCl buffer, pH 7.4), or gentisate (50 mmol/L phosphate buffer, pH 7.4) as the substrate. A certain amount of enzyme was added to initiate the reaction and the absorbance change of substrate or product was recorded by a UV spectrophotometer.

1.8. RT-qPCR assay

Two catechol 1,2-dioxygenase genes (*cat20745* and *cat25170*), which were speculated to be responsible for skatole downstream metabolism, were identified in the genome of strain DMU1. The transcription of *cat20745* and *cat25170* genes in the strain DMU1 in the presence (grew in LB-skatole medium) or absence (grew in LB medium) of skatole were determined by RT-qPCR. Total RNA of the strain DMU1 was isolated using the Bacteria RNA Extraction Kit (Vazyme, China). A reverse transcription-PCR was performed using HiScript II 1st Strand cDNA Synthesis Kit (Vazyme, China), and the resulting cDNAs were quantified with gene-specific primers (**Appendix A Table S1**) using ChamQ SYBR qPCR Master Mix (Vazyme, China) on a real-time PCR system (LightCycler® 480II, Roche, Switzerland). The 16S rRNA gene was used as the reference gene.

1.9. Accession numbers

The metagenome and metatranscriptome sequences have been deposited in the Genome Sequence Archive in BIG Data

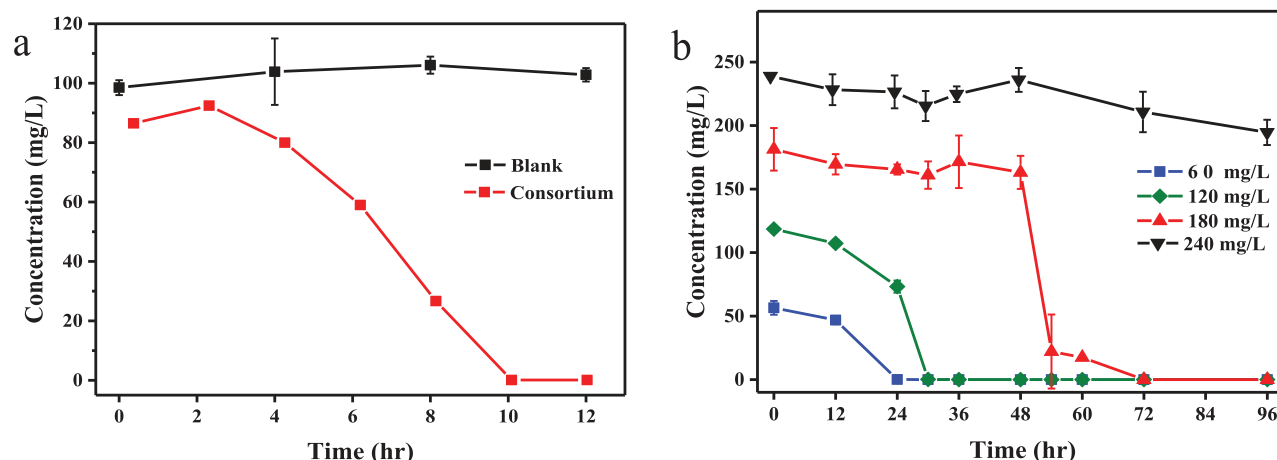

Fig. 1 – Skatole degradation performance of the consortium. Assays in (a) were conducted in MSM with or without the consortium. Assays in (b) were conducted using 2% consortium as the inoculum in MSM with different concentrations of skatole.

Center under accession number CRA004851 (CNCB-NGDC Members and Partners, 2021). The genomic sequences of the strain DMU1 were deposited in the GenBank with accession numbers CP050952.1 and CP050953.1.

2. Results

2.1. *Degradation performance of the skatole-degrading consortium*

The activated sludge previously fed with skatole and glucose in our lab was further domesticated with skatole as the sole carbon source for four months (Ma et al., 2020a). Consequently, a microbial consortium was obtained which could effectively remove 100 mg/L skatole within 10 hr, while nearly no removal of skatole was observed in the control (**Fig. 1a**). Further experiments showed that the microbial community harbored high skatole degradation efficiency under a wide range of temperature (25-40°C) and pH (4.0-10.0) conditions (**Appendix A Fig. S2**). The consortium (2% inoculum size) could completely remove around 60 mg/L and 120 mg/L skatole within 24 hr and 30 hr, respectively (**Fig. 1b**). Skatole was barely degraded when the concentration increased to 240 mg/L, suggesting such a high concentration of skatole was toxic and repressed the growth of microbes in the consortium (Ma et al., 2021).

2.2. *Unraveling the active skatole-degrading populations in the consortium*

The coupled metagenomic and metatranscriptomic analyses were carried out for the consortium. 9.44, 3.86, and 7.43 Gbp quality-filtered reads were generated for samples MS, TS, and TG, respectively (**Appendix A Table S2**). 217,052 contigs (≥500 bp, N50 2523 bp) were obtained through the assembly of metagenomic data. Finally, 486,290 gene catalogues (total length 325 Mbp) were obtained in the MS sample using Linclust, and 257,539 gene catalogues were mapped in metatranscriptomic groups (**Appendix A Table S3**).

The major phyla were Proteobacteria (35.23%) and Actinobacteria (14.64%) by metagenomic analysis, and the ratio changed to 17.80% (Proteobacteria) and 58.57% (Actinobacteria) by metatranscriptomic analysis (**Fig. 2a, Appendix A Table S4**). It suggested that Actinobacteria was active in skatole degradation. The major genera were *Rhodococcus* (8.39%), *Pseudomonas* (4.85%), and *Sphingomonas* (3.98%) by metagenomic analysis (**Fig. 2b, Appendix A Table S5**). However, *Sphingomonas* decreased to the minor population in TS (0.50%) and TG (0.36%), and *Rhodococcus* and *Pseudomonas* increased to the predominant genera (**Fig. 2b**) compared to MS. *Rhodococcus* and *Pseudomonas* occupied 34.69% and 10.48% in TS, which were 4.1- and 2.2-fold higher than the percentages in the MS sample. It suggested that both genera were the active populations related to skatole degradation. The relative percentages of *Rhodococcus* and *Pseudomonas* were 22.37% and 29.57% in the TG sample, respectively. This result indicated that *Pseudomonas* grew better than *Rhodococcus* in the presence of glucose.

2.3. *Identification of the potential oxygenase genes involved in skatole metabolism*

Oxygenases play central roles in aromatic compounds biodegradation through hydroxylation and ring cleavage (Duarte et al., 2014). Skatole was most likely oxidized by unreported oxygenases in the upstream metabolic process, and then the benzene ring was disrupted to enter downstream metabolism (Ma et al., 2021; Fujioka and Wada, 1968). The genes in metagenomic and metatranscriptomic groups were analyzed, and potential oxygenase genes were screened (gene abundance TS/MS>1.5 and TS/TG>1.5). A total of 23 genes were obtained with an up-regulation pattern (**Appendix A Fig. S3a**).

The most up-regulated oxygenase genes in the TS group were the aromatic-ring-hydroxylating dioxygenase (ARHO)

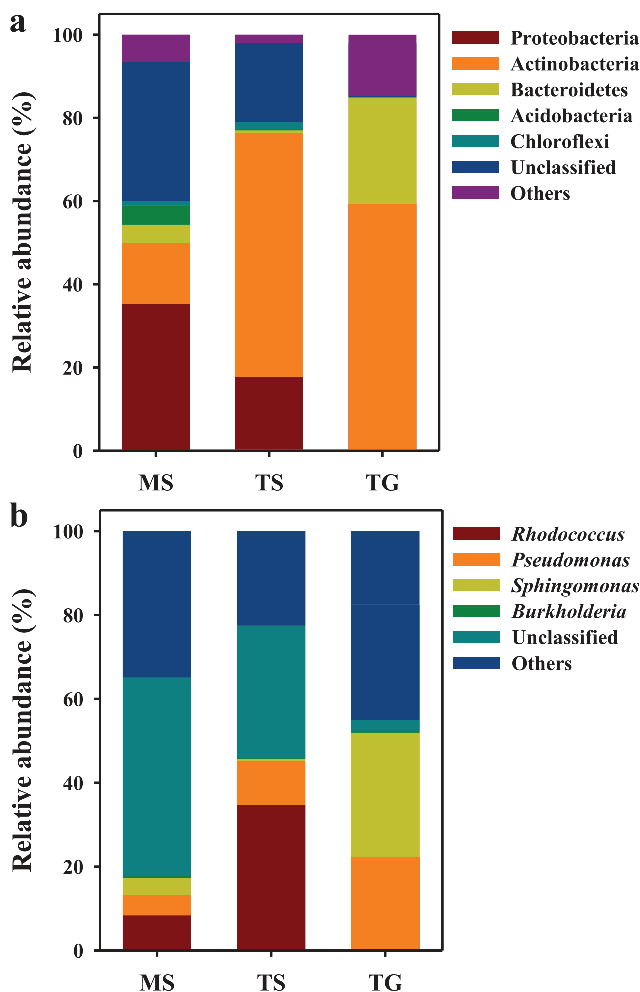

Fig. 2 – Major phyla (a) and genera (b) in the consortium by metagenomic and metatranscriptomic analyses (average abundance more than 1%). MS: metagenomic sequencing of the sample with skatole as sole carbon source. TS: metatranscriptomic sequencing of the sample with skatole as sole carbon source. TG: metatranscriptomic sequencing of the sample with glucose as sole carbon source.

gene α and β subunits. The relative abundances of the α and β subunits reached 0.31 ppm and 0.25 ppm in TS, respectively, which increased 88.1- and 101.6-fold compared to the MS group (**Appendix A Fig. S3a, Appendix A Table S6**). Meanwhile, the expression levels of the two subunit genes in the TS group were 131.6- and 160.0-fold higher than that in the TG group. These results indicated that the ARHO gene was inducible and highly transcribed upon skatole stress. In addition, the putative flavin monooxygenase (FMO) related genes were also enriched and expressed in TS (20.4- and 16.1-fold higher than that in MS and TG, respectively). Both ARHO and flavin monooxygenase were extensively reported in the upstream metabolism of aromatics, suggesting they were likely responsible for skatole transformation (Gao et al., 2021; Qiu et al., 2018). Other oxygenases, including 2,3-dihydroxybiphenyl 1,2-dioxygenase, biphenyl 2,3-dioxygenase, cyclohexanone monooxygenase, nitric oxide dioxygenase, and taurine dioxygenase, were also enriched especially in TS group, and their contribution to skatole degradation also deserved attention.

2.4. *Skatole downstream metabolism is mainly through the catechol pathway in the consortium*

The KEGG pathways of the skatole degradation consortium were analyzed. Among them, a total of 36 KEGG pathways were enriched in the TS compared to the MS and TG samples (**Appendix A Fig. S3b**). Based on the function of these pathways, 8 of which were potentially related to the aromatic biodegradation (**Appendix A Fig. S4**). Further analysis indicated that genes responsible for benzoate, toluene, benzene, phenol, and (methyl)catechol degradation were identified. Several aromatics downstream metabolism genes were actively transcribed, among which the functional genes for catechol metabolism were most abundant in the TS group.

Both ortho-cleavage (C12O pathway) and meta-cleavage (C23O pathway) of catechol degradation pathways were identified in the consortium (**Fig. 3**), and all genes encoding the two pathways were detected. Among these genes, the catechol 2,3-dioxygenase and catechol 1,2-dioxygenase genes were the biomarker genes initiating catechol degradation. The expression level of the catechol 2,3-dioxygenase gene in the TS sample was 10.4- and 50.1-fold higher compared to those in MS and TG, respectively. The relative abundances of the catechol 1,2-dioxygenase gene in the TS sample were 2.3- and 5.1-fold higher compared to those in MS and TG, respectively. In C23O metabolism pathway, catechol could be converted via the 2-hydroxymuconate-semialdehydehydrolase (encoded by *dmpD*) and/or an alternative 2-hydroxymuconate-6-semialdehydedehydrogenase, 4-oxalocrotonate tautomerase, and 2-oxo-3-hexenedioate decarboxylase (*dmpC-praC-dmpH*). It was shown that nearly all catalytic genes in C23O pathways were highly transcribed in TS compared to MS and TG groups. However, in the C12O pathway, the expression levels of genes encoding muconolactone isomerase (*catC*), 3-oxoadipate enol-lactone hydrolase (*pcaDL*), and acetyl-CoA acyltransferase (*fadA*) in TS were lower than those in MS.

2.5. *Reconstruction of metagenome-assembled genomes*

The data of MS was used to assemble genomes of members in the skatole degradation consortium. Eight near-complete genomes (estimated completeness > 90%) with low contamination (< 5%) were retrieved from the assembly (**Table 1**). Among them, Bin9 and Bin51 showed the phylogenetic affiliation to *Pseudoclavibacter*. Bin11, Bin12, Bin35, Bin39, Bin41, and Bin46 were strains of *Pseudomonas, Sphingomonas, Pseudoxanthomonas, Rhodococcus, Allosphingosinicella*, and *Raineyella*, respectively. The mapping reads ratios of Bin39 (*Rhodococcus*) were 7.8% and 5.3% in the TS and TG, respectively, and that for Bin11 (*Pseudomonas*) were 1.9% and 5.1% (**Fig. 4**). The mapping reads ratios of other assembled bins were lower than Bin39 and Bin11. These results were consistent with the findings of active skatole degradation populations (**Fig. 2**), suggesting that *Rhodococcus* and *Pseudomonas* were the main active microbes for skatole degradation.

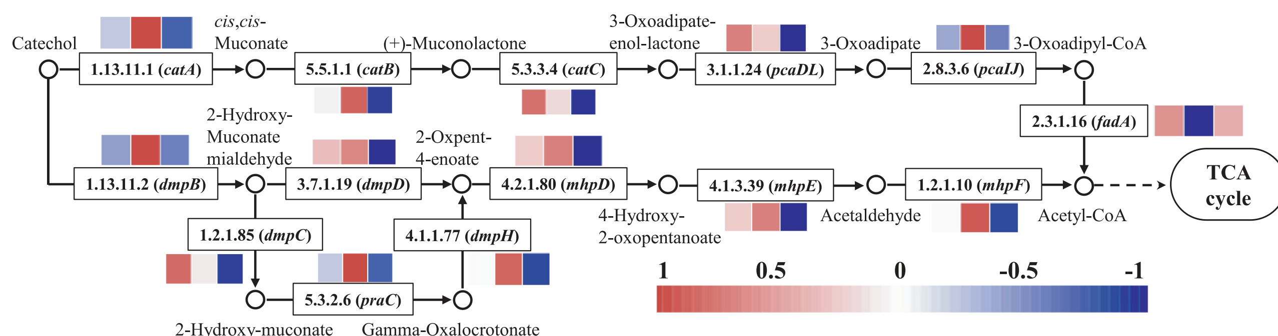

Fig. 3 – Differential expression of genes involved in the catechol metabolism pathway. Enzymes were catechol 1,2-dioxygenase (encoded by *catA*), muconate cycloisomerase (*catB*), muconolactone isomerase (*catC*), 3-oxoadipate enol-lactone hydrolase (*pcaDL*), succinyl CoA:3-oxoadipate CoA transferase (*pcaIJ*), catechol 2,3-dioxygenase (*dmpB*), 2-hydroxymuconate-6-semialdehydedehydrogenase (*dmpC*), 2-hydroxymuconate-semialdehyde hydrolase (*dmpD*), 4-oxalocrotonate tautomerase (*praC*), 2-oxo-3-hexenedioate decarboxylase (*dmpH*), 2-keto-4-pentenoate hydratase (*mhpD*), 4-hydroxy 2-oxovalerate aldolase (*mhpE*), and acetaldehyde dehydrogenase (*mhpF*). Differences in enzyme abundance in MS or activity in TS/TG (Z-trans) are shown in the color squares. The location of squares in each enzyme with the order left to right for MS, TS, and TG, respectively.

Table 1 – Metagenome assembled genomes of the bacteria in the consortium.

ID	Completeness (%)	Contamination (%)	Taxonomy	Total length (Mbp)
Bin9	94.35	3.43	*Pseudoclavibacter*	2.9
Bin11	98.29	1.25	*Pseudomonas*	5.8
Bin12	99.57	0.43	*Sphingomonas*	3.3
Bin35	97.01	0.81	*Pseudoxanthomonas*	2.7
Bin39	98.81	0.57	*Rhodococcus*	5.8
Bin41	98.70	0.16	*Allosphingosinicella*	2.8
Bin46	99.22	0.69	*Raineyella*	3.3
Bin51	91.49	1.17	*Pseudoclavibacter*	3.1

2.6. *Correlating skatole degradation to the individual bacterial species*

Sequence analysis indicated that the majority of the proposed functional genes were in these assembled bins (**Fig. 4**). It was shown that Bin39 (*Rhodococcus*) and Bin46 (*Raineyella*) contained both upstream and downstream genes. The difference was skatole could be degraded via both C23O and C12O pathways in *Rhodococcus*, while *Raineyella* only contained C23O pathways. *Raineyella* represented a novel bacterial re-

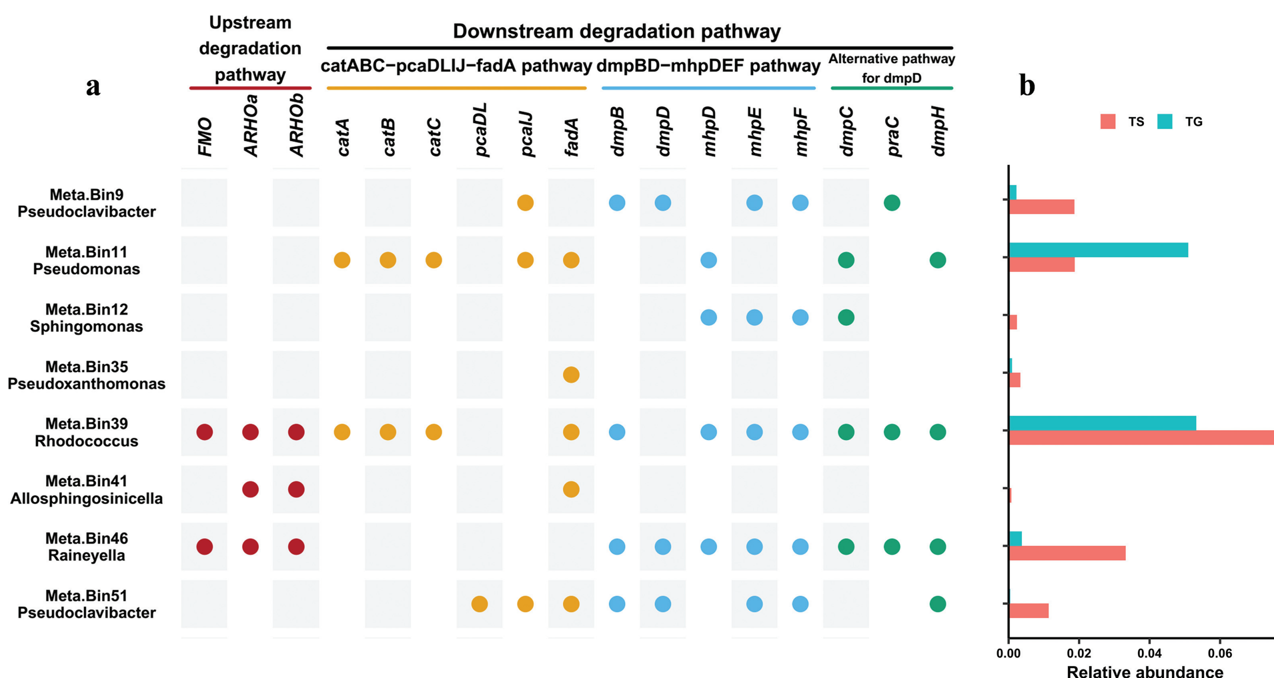

Fig. 4 – Functional gene distribution in assembled bins (a) and relative abundance of the bins (b). FMO: potential flavin monooxygenase. ARHO: aromatic-ring-hydroxylating dioxygenase.

source for skatole degradation which has never been reported before. Bin11 (*Pseudomonas*) lacked upstream oxygenase genes but contained downstream genes, suggesting *Pseudomonas* could utilize the intermediates and facilitate the skatole mineralization in the consortium. Bin9 (*Pseudoclavibacter*) and Bin51 (*Pseudoclavibacter*) contained a partial C12O pathway but lacked upstreaming oxygenase genes, suggesting they could not participate in the skatole metabolism or possess other alternative genes for skatole degradation. Bin35 (*Pseudoxanthomonas*) and Bin41 (*Allosphingosinicella*) might not be directly involved in skatole degradation, or they might degrade skatole via a secondary route.

2.7. Confirmation of the skatole degradation ability by pure Rhodococcus strain

We have tried to screen skatole-degrading strains from the consortium, and a red pure colony was obtained and identified to be *Rhodococcus* sp. DMU1. It was Gram-positive and 1-5 μm in length by scanning electron microscopic analysis (**Appendix A Fig. S5, Fig. 5a**). Evolutionary analysis based on the 16S rRNA gene sequence indicated that the strain DMU1 was closely related to *Rhodococcus aetherivorans* (**Fig. 5b**). The strain DMU1 could utilize skatole as the sole carbon source for growth, which completely degraded 25 mg/L skatole within 36 hr (**Fig. 5c**).

The complete genome of the strain DMU1 was sequenced. The genome size was 6,835,256 bp with a GC content of 70.0%. There were one chromosome (6,119,238 bp) and one plasmid (716,018 bp), which encoded 6181 protein-coding sequences and 69 RNAs (**Appendix A Fig. S6, Appendix A Table S7**). Genome sequence alignment showed that the strain DMU1 had extremely high similarity with Bin39 (sequence similarity >99%), confirming that Bin39 (*Rhodococcus*) was involved in skatole degradation. The average nucleotide identity (ANI) analysis showed that the strain DMU1 was close to *Rhodococcus aetherivorans* (ANI >95%, **Appendix A Table S8**). The strain DMU1 was further confirmed to belong to the species *Rhodococcus aetherivorans* by genome comparison using the Type (Strain) Genome Server (**Appendix A Fig. S7**).

2.8. Identification of a possible gene cluster for skatole upstream metabolism

Based on the genome information of the strain DMU1, a gene cluster containing both ARHO and FMO genes was identified (**Appendix A Table S9**). The ARHO α (1284 bp) and β units (591 bp) were adjacent, showing the highest similarity to 2-halobenzoate 1,2-dioxygenase large subunit from *Burkholderia cepacia* (34.8%) and p-cumate 2,3-dioxygenase small unit from *Pseudomonas putida* (36.3%), respectively. In the gene cluster, a ferredoxin-NADP reductase gene and a ferredoxin gene were found near the ARHO gene. ARHOs are multi-component (two or three) enzymes, consisting of an oxygenase (catalyzing function) and an electron transport chain (ETC, transferring electrons to oxygenase molecules) (Peng et al., 2010). The ETC is composed of either a flavoprotein reductase or a flavoprotein reductase and a ferredoxin. It is suggested that the novel ARHO might belong to a typical three-component enzyme system. The possible FMO showed 25.1% similarity to styrene monooxygenase StyA from *Pseudomonas* sp. (Otto et al., 2004), indicating it might be a novel monooxygenase. A flavin reductase gene was located 7.1 kb far from the *fmo*. However, the relationship between the reductase and FMO required further verification.

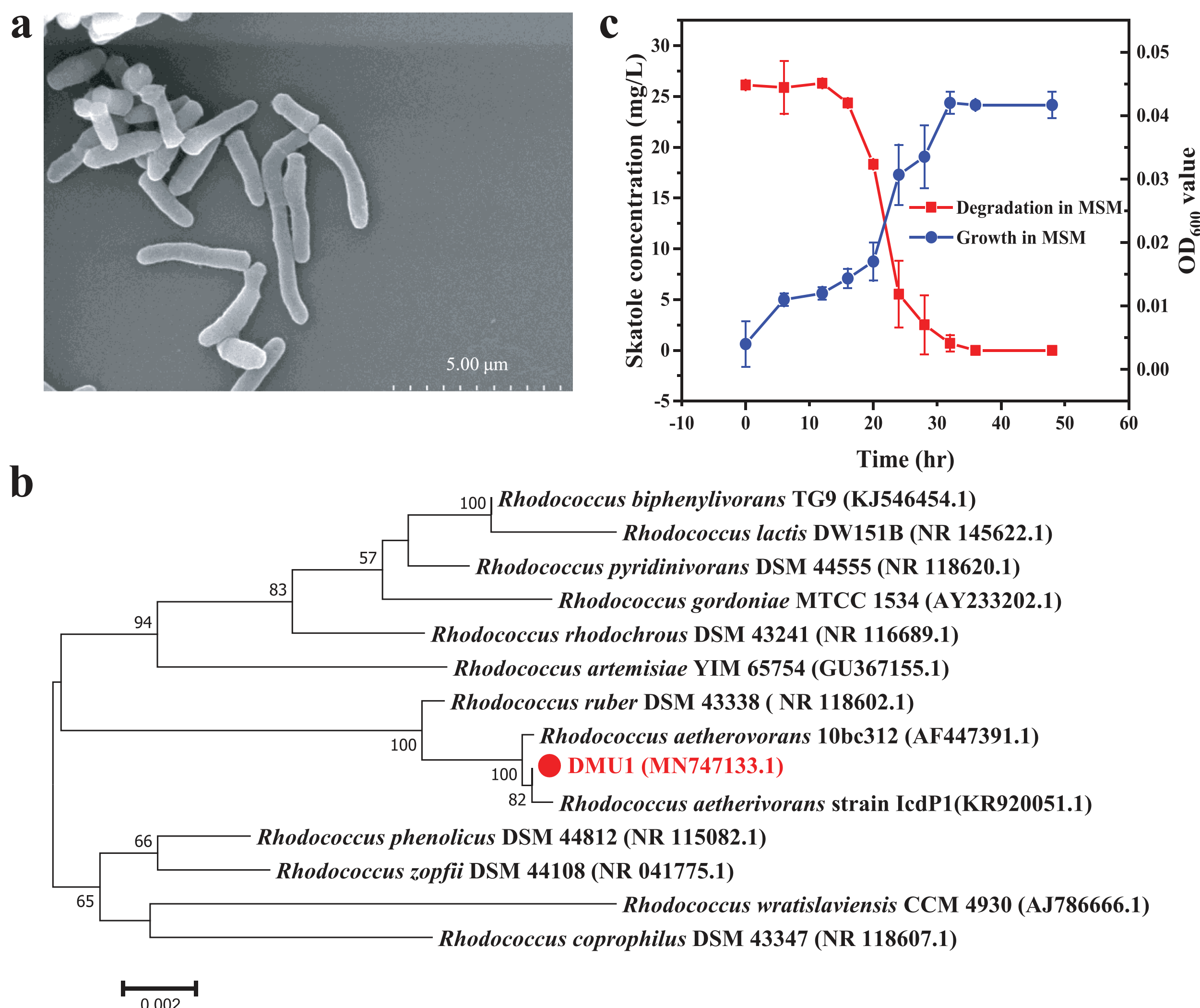

Fig. 5 – Characterization of the strain DMU1. (a) Cell morphology observed by scanning electron microscopy; (b) Phylogenetic tree of the strain DMU1 and related strains; (c) Skatole degradation and growth curves of the strain DMU1 in MSM with skatole as the sole carbon source.

2.9. *Skatole degradation is mainly through the C12O pathway in the strain DMU1*

The strain DMU1 could utilize catechol, protocatechuate, or gentisate as the sole carbon for growth. This result was not surprising since the strain DMU1 contained several central metabolic genes (**Appendix A Table S10**). However, crude enzyme extracts prepared from the strain DMU1 grown on LB and LB-skatole only exhibited C12O activity, and no obvious C23O, P23O, P34O, P45O, and G12O activities were observed (**Appendix A Fig. S8**). The specific activities of C12O from the extracts of LB and LB-skatole grown cells were 0.05±0.01 U/mg and 0.37±0.04 U/mg, respectively. Two catechol 1,2-dioxygenase genes, i.e., *cat20745* (849 bp) and *cat25170* (852 bp), were identified in the strain DMU1 by genome analysis (**Appendix A Table S10**). The expression level of gene *cat20745* was similar in both groups (LB and LB-skatole) quantified by RT-qPCR (**Fig. 6**), and gene *cat25170* was significantly up-regulated (4.2-fold) in the presence of skatole. This result suggested that *cat25170* was an inducible gene that should play a major role in skatole degradation.

3. Discussion

Microbial conversion studies of skatole can be dated back to 50 years ago, and several aerobic bacterial strains have been isolated with skatole-degrading capacity by culture-dependent studies (Ma et al., 2021). However, the degradation in the environment is performed by the microbial community rather than pure strains. Skatole is widespread in swine manure, animal feces, sewage sludges, and wastewater, and the concentration can even reach dozens of mg/L or mg/kg (Ma et al., 2021). Therefore, the enrichment consortia in the current study can be used for skatole bioremediation in practical applications. Meanwhile, this is the first study unveiling the active population for skatole degradation using integrated omics

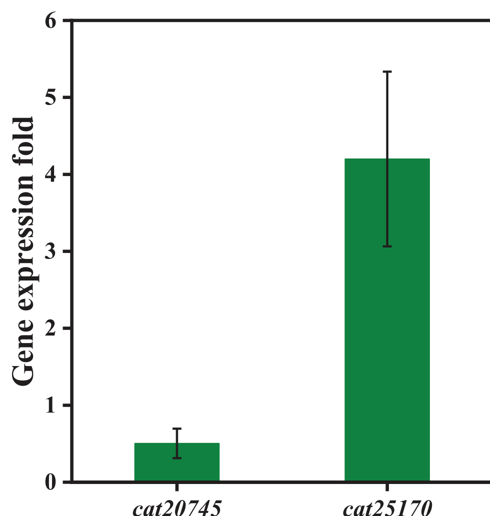

Fig. 6 – RT-qPCR analysis of the two catechol 1,2-dioxygenase genes *cat20745* and *cat25170* in strain DMU1.

and culture-dependent techniques to the best of our knowledge.

Two genera, i.e., *Rhodococcus* and *Pseudomonas* were found to be the major populations in the consortium. Both genera occupied much higher proportions in metatranscriptomic data compared to metagenomic data, suggesting they were active in the skatole-degrading process. *Rhodococcus* is a well-studied aromatic-degrading genus with diverse ecological niches (Martínková et al., 2009; Gao et al., 2020). The large genomes and redundant catabolic pathways ensure its high efficiency in aromatic degradation. Fortunately, we have successfully isolated the strain *Rhodococcus aetherivorans* DMU1, i.e., the assembled Bin39 in the consortium, and proved it was capable of degrading skatole. Overall, the skatole degradation performance of the consortium was superior to the pure strain DMU1 (**Figs. 1, 5c**), implying other strains facilitated the skatole removal process. *Pseudomonas* was one of the most investigated and well-known aromatic-degrading genera in the lab (Volke et al., 2020; Hu et al., 2019). *Pseudomonas aeruginosa* GS and *Pseudomonas putida* LPC24 were previously reported with skatole degradation capacity (Yin et al., 2006; Li et al, 2010). However, we did not obtain *Pseudomonas* strains using skatole as the sole carbon source in this study. These results inferred that *Pseudomonas* might participate in skatole degradation but lacked the complete metabolic pathway, or the conditions used for the experiments of *Pseudomonas* were not suitable for skatole degradation.

The enzymes responsible for skatole initial metabolism remain unclear to date (Ma et al., 2021). Integrated metagenomic and metatranscriptomic analyses identified two possible oxygenase genes, i.e., an aromatic-ring-hydroxylating dioxygenase gene and a putative flavin monooxygenase gene, which were highly expressed in TS compared to MS and TG group (**Appendix A Fig. S3**). Both genes were identified in the assembled Bin39. Further analysis of the strain DMU1 genome showed that they were in a gene cluster (**Appendix A Table S9**). The aromatic ring-hydroxylating dioxygenase gene should contain three components, including the oxygenase (α and β subunits), a ferredoxin–NADP reductase, and a ferredoxin gene component. They constitute a typical ARHO, which has been widely investigated to be involved in aromatics biodegradation, especially for the first oxidation step (Gao et al., 2020; Li et al., 2021; Chen et al., 2014, 2017). The putative flavin monooxygenase showed certain identities to previously reported styrene monooxygenase and indole oxygenases (Otto et al., 2004; Paul et al., 2021; Tischler et al., 2009; Ma et al., 2018; Qu et al., 2017; Sadauskas et al., 2017). Based on the structural similarity of skatole and indole, the FMO was also speculated to be responsible for skatole oxidation. Further transcriptomic response of the strain DMU1 to skatole stress and gene function studies were required to elucidate the exact roles of the ARHO and FMO. However, we also could not rule out the possibility that the expression of functional genes for skatole degradation was constitutive, and the genes were not identified in this study.

The central metabolic pathways of aromatics were conservative, and hydroxylated products were mainly catechol, protocatechuate, gentisate, and homogentisate (Haritash and Kaushik, 2009; Fuchs et al., 2011). Our results indicated that

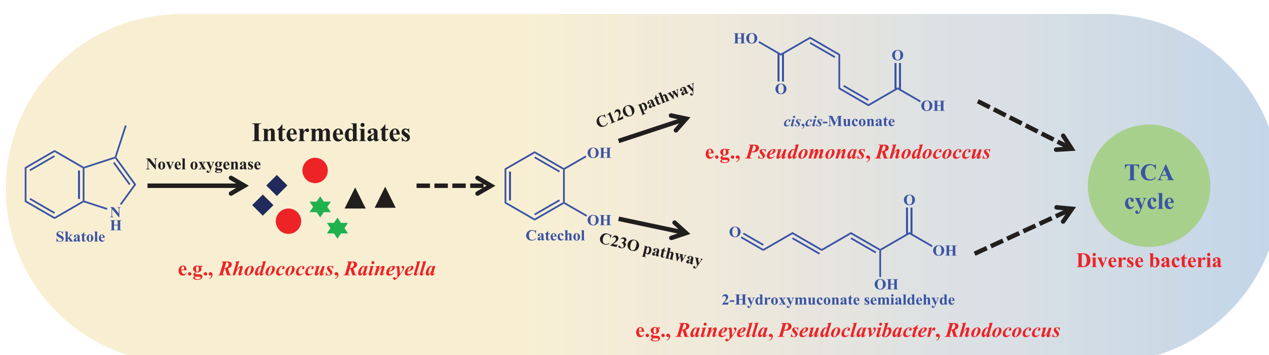

Fig. 7 – A proposed synergistic consortium involved in skatole degradation. *Rhodococcus* and *Raineyella* containing predicted novel oxygenases were involved in upstream metabolism. *Pseudomonas* and *Rhodococcus* were responsible for C12O downstream metabolism. *Raineyella*, *Pseudoclavibacter*, and *Rhodococcus* were responsible for C23O downstream metabolism. TCA: tricarboxylic acid cycle.

skatole was mainly degraded via catechol pathways, and both C12O and C23O genes were highly enriched and expressed in the consortium. *Pseudoclavibacter, Rhodococcus*, and *Raineyella* contained C23O genes, and *Rhodococcus* and *Pseudomonas* contained C12O genes. Overall, the gene abundance of C23O was higher than the C12O, and all genes encoding the C23O pathway were up-regulated in the TS group compared to TG, demonstrating that C23O was the main pathway in the consortium. As for *Rhodococcus* sp. DMU1, the enzymatic and RT-qPCR assays showed that only the C12O pathway was activated in the flask experiment. This result implied that the skatole central degradation was catalyzed by various strains rather than *Rhodococcus* in the community. Another possibility was the different culture conditions for strain DMU1 in pure strain and community studies could result in different metabolic strategies.

It has been observed in many studies that pollutants degradation in the consortium was usually more efficient than a single strain, and non-degraders could enhance the degradation performance (Qi et al., 2021; Liu et al., 2019; Hu et al., 2020; Yu et al., 2019). Based on the results of integrated meta-omics and pure culture study, the skatole metabolic process involved in the syntrophic associations of the skatole-degrading consortium was proposed as shown in **Fig. 7**. Although *Rhodococcus* was an effective and active degrader, the complete and efficient degradation of skatole should be performed by the mixture of diverse bacteria, including *Pseudomonas, Pseudoclavibacter*, and *Raineyella*, which might be the underlying mechanism of the community and functionality stability. This study reveals hitherto systematically molecular insights into the understanding of the skatole degradation process, which can facilitate the bioremediation strategy development by constructing the synthetic community in practical application.

4. Conclusions

An efficient skatole-degrading consortium was domesticated using skatole as the sole carbon source. We identified *Rhodococcus* as the dominant and active species for skatole degradation by integrated metagenomics, metatranscriptomics, and culture-dependent function verification. Some other genera, such as *Pseudomonas, Pseudoclavibacter*, and *Raineyella*, should cooperate with *Rhodococcus* for function stability. The aromatic-ring-hydroxylating dioxygenase gene and flavin monooxygenase gene were identified in the consortium, which might be related to skatole upstream metabolism. The skatole downstream degradation was mainly via the catechol pathway in the consortium. Furthermore, a pure strain *Rhodococcus* sp. DMU1 which could utilize skatole as the sole carbon source was isolated from the consortium. The complete genome sequence of strain DMU1 was obtained and proved to be the major strain in the consortium. Further enzymatic and RT-qPCR assays verified that skatole was degraded via the catechol ortho-cleavage pathway. Overall, we demonstrate that integrated metagenomic, metatranscriptomic, and pure culture techniques are useful to unravel the functional populations and genes for pollutants biodegradation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jes.2022.06.025.

REFERENCES

Achermann, S., Mansfeldt, C.B., Müller, M., Johnson, D.R., Fenner, K., 2019. Relating metatranscriptomic profiles to the micropollutant biotransformation potential of complex microbial communities. Environ. Sci. Technol. 54, 235–244.

Beveridge, T.J., 2001. Use of the Gram stain in microbiology. Biotech. Histochem. 76, 111–118.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.

Bushnell, B., 2014. BBMap: A fast, accurate, splice-aware aligner. Lawrence Berkeley National Lab (LBNL), Berkeley, CA (United States).

Chen, X., Ji, J., Zhao, L., Qiu, J., Dai, C., Wang, W., et al., 2017. Molecular mechanism and genetic determinants of buprofezin degradation. Appl. Environ. Microbiol. 83 e00868–17.

Chen, Q., Wang, C.H., Deng, S.K., Wu, Y.D., Li, Y., Jiang, J.D., et al., 2014. Novel three-component Rieske non-heme iron oxygenase system catalyzing the *N*-dealkylation of chloroacetanilide herbicides in *Sphingomonads* DC-6 and DC-2. Appl. Environ. Microbiol. 80, 5078–5085.

Chin, C.S., Alexander, D.H., Marks, P., Klammer, A.A., Drake, J., Heiner, C., et al., 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat. Methods 10, 563–569.

CNCB-NGDC Members and Partners, 2021. Database resources of the national genomics data center, China national center for bioinformation in 2021. Nucleic Acids Res. 49, D18–D28.

Duarte, M., Jauregui, R., Vilchez-Vargas, R., Junca, H., Pieper, D.H., 2014. AromaDeg, a novel database for phylogenomics of aerobic bacterial degradation of aromatics. Database 2014, 1–12.

Fuchs, G., Boll, M., Heider, J., 2011. Microbial degradation of aromatic compounds—from one strategy to four. Nat. Rev. Microbiol. 9, 803–816.

Fujioka, M., Wada, H., 1968. The bacterial oxidation of indole. Biochim. Biophys. Acta Gen. Subj. 158, 70–78.

Fukuoka, K., Ozeki, Y., Kanaly, R.A., 2015. Aerobic biotransformation of 3-methylindole to ring cleavage products by *Cupriavidus* sp. strain KK10. Biodegradation 26, 359–373.

Gao, Y.Z., Liu, X.Y., Liu, H., Guo, Y., Zhou, N.Y., 2020. A Bph-like nitroarene dioxygenase catalyzes the conversion of

3-nitrotoluene to 3-methylcatechol by *Rhodococcus* sp. strain ZWL3NT. Appl. Environ. Microbiol. 86 e02517–19.
Gao, Y.Z., Palatucci, M.L., Waidner, L.A., Li, T., Guo, Y., Spain, J.C., et al., 2021. A Nag-like dioxygenase initiates 3, 4-dichloronitrobenzene degradation via 4,5-dichlorocatechol in *Diaphorobacter* sp. strain JS3050. Environ. Microbiol. 23, 1053–1065.
Haritash, A.K., Kaushik, C.P., 2009. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J. Hazard. Mater. 169, 1–15.
Herter, C.A., 1908. The occurrence of skatol in the human intestine. J. Biol. Chem. 4, 101–109.
Hu, B., Wang, M., Geng, S., Wen, L., Wu, M., Nie, Y., et al., 2020. Metabolic exchange with non-alkane-consuming *Pseudomonas stutzeri* SLG510A3-8 improves n-alkane biodegradation by the alkane degrader *Dietzia* sp. strain DQ12-45-1b. Appl. Environ. Microbiol. 86 e02931–19.
Hu, H., Wang, L., Wang, W., Wu, G., Tao, F., Xu, P., et al., 2019. Regulatory mechanism of nicotine degradation in *Pseudomonas putida*. mBio 10 e00602–19.
Hu, S., Liu, G., Zhang, L., Gan, Y., Wang, B., Freilich, S., et al., 2021. A synergistic consortium involved in *Rac*-dichlorprop degradation as revealed by DNA-stable isotope probing and metagenomics analysis. Appl. Environ. Microbiol. 87 e01562–21.
Hyatt, D., Chen, G.L., LoCascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J., 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinf. 11, 1–11.
Kang, D.D., Li, F., Kirton, E., Thomas, A., Egan, R., An, H., et al., 2019. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. PeerJ 7, e7359.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.
Li, D., Liu, C.M., Luo, R., Sadakane, K., Lam, T.W., 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31, 1674–1676.
Li, P., Tong, L., Liu, K., Wang, Y., 2010. Biodegradation of 3-methylindole by *Pseudomonas putida* LPC24 under oxygen limited conditions. Fresenius Environ. Bull. 19, 238–242.
Li, T., Gao, Y.Z., Xu, J., Zhang, S.T., Guo, Y., Spain, J.C., et al., 2021. A recently assembled degradation pathway for 2,3-dichloronitrobenzene in *Diaphorobacter* sp. strain JS3051. mBio 12 e02231–21.
Liu, D., Wei, Y., Liu, X., Zhou, Y., Jiang, L., Yin, J., et al., 2018. Indoleacetate decarboxylase is a glycyl radical enzyme catalysing the formation of malodorant skatole. Nat. Commun. 9, 1–8.
Liu, Z., Huang, H., Qi, M., Wang, X., Adebanjo, O.O., Lu, Z., 2019. Metabolite cross-feeding between *Rhodococcus ruber* YYL and *Bacillus cereus* MLY1 in the biodegradation of tetrahydrofuran under pH stress. Appl. Environ. Microbiol. 85 e01196–19.
Ma, Q., Zhang, X., Qu, Y., 2018. Biodegradation and biotransformation of indole: advances and perspectives. Front. Microbiol. 9, 2625.
Ma, Q., Liu, S., Li, S., Hu, J., Tang, M., Sun, Y., 2020a. Removal of malodorant skatole by two enriched microbial consortia: performance, dynamic, function prediction and bacteria isolation. Sci. Total Environ. 725, 138416.
Ma, Q., Qu, H., Meng, N., Li, S., Wang, J., Liu, S., et al., 2020b. Biodegradation of skatole by *Burkholderia* sp. IDO3 and its successful bioaugmentation in activated sludge systems. Environ. Res. 182, 109123.
Ma, Q., Meng, N., Li, Y., Wang, J., 2021. Occurrence, impacts, and microbial transformation of 3-methylindole (skatole): a critical review. J. Hazard. Mater. 416, 126181.
Martínková, L., Uhnáková, B., Pátek, M., Nešvera, J., Křen, V., 2009. Biodegradation potential of the genus *Rhodococcus*. Environ. Int. 35, 162–177.
Meier-Kolthoff, J.P., Göker, M., 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat. Commun. 10, 1–10.
Meng, X., He, Z.F., Li, H.J., 2013. Purification and characterization of a novel skatole degrading protease from *Lactobacillus brevis* 1.12. Food Sci. Biotechnol. 22, 1–7.
Meyer-Cifuentes, I.E., Werner, J., Jehmlich, N., Will, S.E., Neumann-Schaal, M., Öztürk, B., 2020. Synergistic biodegradation of aromatic-aliphatic copolyester plastic by a marine microbial consortium. Nat. Commun. 11, 1–13.
Nagata, Y., 2003. Measurement of odor threshold by triangle odor bag method. Odor Meas. Rev. 118, 118–127.
Otto, K., Hofstetter, K., Röthlisberger, M., Witholt, B., Schmid, A., 2004. Biochemical characterization of StyAB from *Pseudomonas* sp. strain VLB120 as a two-component flavin-diffusible monooxygenase. J. Bacteriol. 186, 5292–5302.
Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., Tyson, G.W., 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 25, 1043–1055.
Paul, C.E., Eggerichs, D., Westphal, A.H., Tischler, D., van Berkel, W.J., 2021. Flavoprotein monooxygenases: versatile biocatalysts. Biotechnol. Adv. 51, 107712.
Peng, R.H., Xiong, A.S., Xue, Y., Fu, X.Y., Gao, F., Zhao, W., et al., 2010. A profile of ring-hydroxylating oxygenases that degrade aromatic pollutants. Rev. Environ. Contam. Toxicol. 206, 65–94.
Qi, M., Liang, B., Zhang, L., Ma, X., Yan, L., Dong, W., et al., 2021. Microbial interactions drive the complete catabolism of the antibiotic sulfamethoxazole in activated sludge microbiomes. Environ. Sci. Technol. 55, 3270–3282.
Qiu, J., Liu, B., Zhao, L., Zhang, Y., Cheng, D., Yan, X., et al., 2018. A novel degradation mechanism for pyridine derivatives in *Alcaligenes faecalis* JQ135. Appl. Environ. Microbiol. 84 e00910–18.
Qu, Y., Ma, Q., Liu, Z., Wang, W., Tang, H., Zhou, J., et al., 2017. Unveiling the biotransformation mechanism of indole in a *Cupriavidus* sp. strain. Mol. Microbiol. 106, 905–918.
Qu, Y., Shen, E., Ma, Q., Zhang, Z., Liu, Z., Shen, W., et al., 2015. Biodegradation of indole by a newly isolated *Cupriavidus* sp. SHE. J. Environ. Sci. 34, 126–132.
Richter, M., Rosselló-Móra, R., Oliver Glöckner, F., Peplies, J., 2016. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32, 929–931.
Sadauskas, M., Vaitekūnas, J., Gasparavičiūtė, R., Meškys, R., 2017. Indole biodegradation in *Acinetobacter* sp. strain O153: genetic and biochemical characterization. Appl. Environ. Microbiol. 83 e01453–17.
Sharma, N., Doerner, K.C., Alok, P.C., Choudhary, M., 2015. Skatole remediation potential of *Rhodopseudomonas palustris* WKU-KDNS3 isolated from an animal waste lagoon. Lett. Appl. Microbiol. 60, 298–306.
Shen, X.H., Liu, Z.P., Liu, S.J., 2004. Functional identification of the gene locus *ncg 12319* and characterization of catechol 1, 2-dioxygenase in *Corynebacterium glutamicum*. Biotechnol. Lett. 26, 575–580.
Steinegger, M., Söding, J., 2018. Clustering huge protein sequence sets in linear time. Nat. Commun. 9, 1–8.
Tesso, T.A., Zheng, A., Cai, H., Liu, G., 2019. Isolation and characterization of two *Acinetobacter* species able to degrade 3-methylindole. PLoS One 14, 0211275.
Tischler, D., Eulberg, D., Lakner, S., Kaschabek, S.R., van Berkel, W.J., Schlömann, M., 2009. Identification of a novel self-sufficient styrene monooxygenase from *Rhodococcus opacus* 1CP. J. Bacteriol. 191, 4996–5009.

Tremblay, J., Fortin, N., Elias, M., Wasserscheid, J., King, T.L., Lee, K., et al., 2019. Metagenomic and metatranscriptomic responses of natural oil degrading bacteria in the presence of dispersants. Environ. Microbiol. 21, 2307–2319.
Volke, D.C., Calero, P., Nikel, P.I., 2020. *Pseudomonas putida*. Trends Microbiol. 28, 512–513.
Weems, J.M., Cutler, N.S., Moore, C., Nichols, W.K., Martin, D., Makin, E., et al., 2009. 3-Methylindole is mutagenic and a possible pulmonary carcinogen. Toxicol. Sci. 112, 59–67.
Wesoly, R., Weiler, U., 2012. Nutritional influences on skatole formation and skatole metabolism in the pig. Animals 2, 221–242.
Wick, R.R., Judd, L.M., Gorrie, C.L., Holt, K.E., 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13, e1005595.
Yin, B., Huang, L., Gu, J.D., 2006. Biodegradation of 1-methylindole and 3-methylindole by mangrove sediment enrichment cultures and a pure culture of an isolated *Pseudomonas aeruginosa* Gs. Water Air Soil Pollut. 176, 185–199.
Yu, K., Yi, S., Li, B., Guo, F., Peng, X., Wang, Z., et al., 2019. An integrated meta-omics approach reveals substrates involved in synergistic interactions in a bisphenol A (BPA)-degrading microbial community. Microbiome 7, 1–13.
Zhou, N.Y., Fuenmayor, S.L., Williams, P.A., 2001. *nag* genes of *Ralstonia* (formerly *Pseudomonas*) sp. strain U2 encoding enzymes for gentisate catabolism. J. Bacteriol. 183, 700–708.