Bacterial and fungal community compositions and structures of a skatole-degrading culture enriched from pig slurry

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Abstract

In this study, the aerobic activated sludge for skatole removal was enriched from pig slurry in three parallel sequencing batch reactors. The sludge system exhibited a satisfactory performance for skatole removal during the 40 days operation. High-throughput sequencing results showed that the α -diversity remained unchanged before and after the operation process. However, the structures of bacterial and fungal communities notably shifted. Particularly, *Arthrobacter* increased to be the major bacterial genus from $2.15 \pm 0.76\%$ (day 0) to $23.80 \pm 24.36\%$ (day 40), and *Fusicolla* became the major fungal genus from $1.20 \pm 0.48\%$ (day 0) to $37.17 \pm 7.47\%$ (day 40). These results indicated that *Arthrobacter* and *Fusicolla* might participate in skatole removal in sludge systems, though both genera were not reported to be able to degrade skatole. This is the first study describing skatole-degrading bacterial and fungal communities in the enrichment from pig slurry to the best of our knowledge, providing important guidance for skatole control and bioremediation.

Keywords Skatole · Biodegradation · Pig slurry · Bacterial community · Fungal community

Skatole (3-Methylindole), a well-known foul-smelling odorant, is formed by microbial fermentation of tryptophan in the intestine of humans and animals (Mackie et al. 1998). In addition to natural formation, skatole is also generated by the pyrolysis of tryptophan in tobacco combustion (Weems and Yost 2010). Skatole shows selective pulmonary toxicity

to Clara and epithelial cells in humans and ruminants, which can cause acute bovine pulmonary edema and emphysema (Carlson et al. 1972). Skatole can accrete in adipose tissue of pigs despite liver clearance and excretion, being one of the main contributors for boar taint (Mackie et al. 1998). Furthermore, skatole is an oviposition attractant for mosquitoes which may promote diseases transmission (Hughes et al. 2010). Studies have shown that anaerobic bacteria, such as *Clostridium* and *Lactobacillus*, were responsible for skatole production (Liu et al. 2018). Preventing skatole formation in animal guts by altering the microbial community structures and activities is attracting attention, but still impracticable at present.

Biodegradation is a feasible and eco-friendly way to remove skatole. Yin et al. (2006) have reported that *Pseudomonas aeruginosa* Gs isolated from mangrove sediment enrichment could mineralize skatole. Afterwards, *Lactobacillus brevis*, *Rhodopseudomonas palustris*, *Cupriavidus* sp., *Acinetobacter* spp. and *Burkholderia* sp., were successively reported to degrade skatole (Meng et al. 2013; Sharma et al. 2015; Fukuoka et al. 2015; Tesso et al. 2019; Ma et al. 2019, 2020a). Most of the reported strains belonged to Gram-negative bacteria, and fungi have yet been investigated so far. Pure strains may not survive in practical environments, thus the microbial communities responsible for skatole

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degradation deserve attention, which, however, have rarely been explored.

Activated sludge system is the most common strategy for wastewater treatment. Therefore, the aerobic sequencing batch reactors (SBRs) were constructed in this study. Fresh pig slurry collected from Pulandian (Dalian, China) was acclimated in flasks using mineral salt medium (MSM) for almost a month (Ma et al. 2020b). Then the sludge was transferred to three parallel SBRs with mixed liquor suspended solids (MLSS) of 6400 mg/L for enrichment. The whole enrichment process was divided into two stages, including a low skatole concentration stage (10 mg/L skatole, day 1–20) and a high skatole concentration stage (50 mg/L skatole, day 21–40). The residual skatole in the effluent was recorded daily (Fig. 1). In the first stage, the sludge removed a half of skatole in the first two days, and the effluent skatole concentration gradually dropped to zero after five days of operation. In the second stage, the influent skatole sharply increased to 50 mg/L. The sludge showed

a great adaptability, and the skatole in effluent was almost undetectable after three days fluctuation, suggesting that the skatole-degrading community had been enriched. Gu et al. (1992) has reported that anaerobic activated sludge removed 0.1 mM (13.1 mg/L) skatole over 32 days, whose removal rate was significantly lower than that in this study (50 mg/L in one day). In addition, the removal efficiency of the enrichment culture was also superior to that of pure strains, such as *Pseudomonas aeruginosa* Gs (65.5 mg/L in 12 days) (Yin et al. 2006), *Lactobacillus brevis* 1.12 (65% of 1.0 mg/L in 120 h) (Meng et al. 2013), and *Rhodopseudomonas palustris* WKU-KDNS3 (93% of 13.1 mg/L in 21 days) (Sharma et al. 2015).

Sludge samples on day 0 and day 40 were collected for DNA extraction. The bacterial 16S rRNA V4 region and fungal internal transcribed spacer (ITS) were amplified. The clean PCR products with different barcodes were sequenced on Illumina sequencing platform. Sample information was provided in Table 1. The raw sequence data of amplicon

Fig. 1 Skatole concentration in the effluent during the enrichment process. The SBRs were operated 24 h for a cycle. Influent skatole was 10 mg/L from day 1 to day 20, and skatole concentration increased to 50 mg/L from day 21 to day 40. The effluent skatole was determined every day

Table 1 Sample information and primers for sequencing

Sample	Forward barcode	Forward primer	Reverse primer
Z1_B0	GTCCGTACCTTG	GTGCCAGCMGCCGCGGTAA	GGACTACHVGGGTWTCTAAT
Z2_B0	AACGCATGAGGA		
Z3_B0	CTGTTGACTGAC		
Z1_B40	CTGGTCAGACGA		
Z2_B40	TCTCGATAGGCT		
Z3_B40	TCTATGGTACAG		
Z1_F0	AGCATATGAGAG	GTGARTCATCGARTCTTTG	TCCTCCGCTTATTGATATGC
Z2_F0	AGGCTACACGAC		
Z3_F0	AAGCTGCAGTCG		
Z1_F40	AATCAGTCTCGT		
Z2_F40	ACGACGTCTTAG		
Z3_F40	ACGTGAGAGAAT		

Z1, Z2 and Z3 represent the three biological replicates, B bacteria, F fungi, 0: day 0, 40: day 40

sequencing have been deposited in the Genome Sequence Archive in BIG Data Center (Zhang et al. 2019). The high-throughput sequencing raw data were analyzed on the galaxy pipeline as described previously (Ma et al. 2020b; Feng et al. 2017). In total, 754,735 (bacteria) and 904,405 (fungi) clean reads were generated, which were assigned to 5756 and 684 OTUs for bacteria and fungi, respectively. The rarefaction curves indicated that the sequences were enough to reach saturation (Fig. S1), which were resampled at 76,383 and 55,800 for bacteria and fungi, respectively, before following analyses.

The microbial richness and diversity were calculated as shown in Fig. S2. As for bacteria, the Shannon index values of on day 0 and day 40 were 2.97 ± 0.06 and 3.31 ± 0.36 , respectively. The Inverse Simpson index values were 6.09 ± 0.74 (day 0) and 7.58 ± 3.91 (day 40), and the observed richness were 1518 ± 56 (day 0) and 1475 ± 26 (day 40). The above values did not change significantly between day 0 and day 40 (Student's *t* test, $P > 0.05$). As for fungi, the average values of Shannon index, Inverse Simpson index, and observed richness were 2.49 ± 0.32 , 6.53 ± 2.95 , 234 ± 28 for day 0, and 2.10 ± 0.22 , 4.86 ± 0.69 , 206 ± 29 for day 40, and there were also no significant changes between day 0 and day 40 (Student's *t* test, $P > 0.05$). It suggested that skatole addition did not affect the sludge microbial richness and diversity. The Shannon index and Inverse Simpson index values of fungi were similar to those of bacteria, while observed richness values were much lower.

It was demonstrated that both bacterial and fungal communities on day 40 were separated from those of day 0 by principal component analysis (Fig. S3). To further elaborate the changes, microbial community structures at phylum and genus levels were analyzed (Fig. 2).

For bacteria, it was observed that Proteobacteria and Bacteroidetes were the predominant phyla on day 0 and 40 (Fig. S4). After skatole acclimation, Proteobacteria decreased

from $69.22 \pm 8.50\%$ to $31.98 \pm 5.03\%$, while Bacteroidetes increased from $23.03 \pm 8.26\%$ to $37.87 \pm 19.82\%$. It was noted that Actinobacteria was significantly enriched from 2.92 ± 0.63 to $25.21 \pm 20.51\%$. At genus level (Fig. 2a), *Flavobacterium* was the preponderant genus on both day 0 ($21.56 \pm 8.01\%$) and day 40 ($14.13 \pm 9.39\%$). *Arthrobacter*, *Thauera*, *Methylophilus* and *Zoogloea* become the major genera in the enrichment culture, increasing from $2.15 \pm 0.76\%$, $0.32 \pm 0.03\%$, $0.25 \pm 0.03\%$ and $0.56 \pm 0.04\%$ to $23.80 \pm 24.36\%$, $7.92 \pm 8.11\%$, $7.55 \pm 4.85\%$ and $4.35 \pm 1.58\%$, respectively. *Arthrobacter* spp. were effective bacteria with the ability to degrade many kinds of persistent environmental pollutants, including triazines, organophosphorus, and benzenes (Fu et al. 2014). In a previous study, Ma et al. (2020b) also found that *Arthrobacter* was enriched in the sludge derived from the topsoil of cattle and goose houses, further implying that *Arthrobacter* played an important role in skatole removal. However, previously reported skatole-degrading genera, such as *Pseudomonas*, *Burkholderia* and *Cupriavidus* were not significantly enriched.

For fungi, Ascomycota ($23.86 \pm 11.35\%$), Mucoromycota ($50.46 \pm 22.59\%$), and Basidiomycota ($24.81 \pm 9.19\%$) were the predominant phyla in the original sludge. After enrichment, Ascomycota ($42.92 \pm 7.64\%$) and Basidiomycota ($12.82 \pm 3.21\%$) were still the dominate phyla, while Mucoromycota ($0.73 \pm 0.28\%$) almost vanished. On the contrary, Ciliophora was enriched to the major population ($41.14 \pm 11.40\%$). At the genus level, *Mucor* was the most abundant genus in the initial sludge, with an abundance of $50.46 \pm 22.59\%$, which sharply reduced to $0.73 \pm 0.28\%$ on day 40. Another major genus *Trichosporon* ($9.89 \pm 5.66\%$) also disappeared on day 40. It was noted that *Fusicolla* drastically increased from $1.20 \pm 0.48\%$ to $37.17 \pm 7.47\%$ after enrichment. *Fusicolla* has generally been considered as a synonym of *Fusarium* (Gräfenhan et al. 2011), an important plant pathogen with a worldwide distribution, which

Fig. 2 Abundance of the major bacteria (a) and fungi (b) at the genera level. The top 10 genera were chosen. Z1, Z2 and Z3 represent the three biological replicates, B bacteria, F fungi, 0: day 0, 40: day 40.

For example, Z1_B0, Z2_B0 and Z3_B0 mean the bacterial information of the three parallel reactors at day 0

possessed superior degradation capacity towards many compounds, including lignin, benzo[a]pyrene and various aromatics (Morales et al. 2017). Since the skatole degradation by fungi has not been reported so far, further studies on skatole removal by fungi (such as *Fusicolla* and *Fusarium*) are worth expecting.

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Author contributions QH, MN and LS performed the experiments, data collection and statistical analysis. WJ and SY contributed suggestions. MQ designed the study, contributed to analyzing data, and wrote the manuscript.

Data availability The raw sequence data have been deposited in the Genome Sequence Archive in BIG Data Center with accession number CRA002571.

Compliance with ethical standards

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

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