Ye and Fu *Annals of Microbiology* (2023) 73:22
https://doi.org/10.1186/s13213-023-01722-x

Annals of Microbiology

ORIGINAL ARTICLE

Open Access

Screening of skatole-degrading bacteria and control of human fecal odor by compound bacteria

Jiangyu Ye[1,2*] and Qian Fu[1,2]

Abstract

Purpose The biodegradation of skatole was used as a starting point in this study, and existing strains of degrading NH_3 and H_2S in the laboratory were combined to create a composite deodorant. The deodorization effect of the composite deodorant on human feces was investigated in order to provide a foundation for fecal odor gas treatment.

Methods Skatole-degrading bacteria were identified, degradation conditions were optimized, and skatole metabolites were identified using liquid chromatography-mass spectrometry (LC-MS). The skatole-degrading bacteria were combined with the existing strains of degrading NH_3 and H_2S in the laboratory to make a compound deodorizer, and the deodorizing effect of the compound deodorizer on fresh human feces and old human feces was compared. Liquid chromatography, specific sensors, and gas chromatography were used to determine the contents of skatole, NH_3, H_2S, and volatile organic compounds, and the microbial diversity was examined.

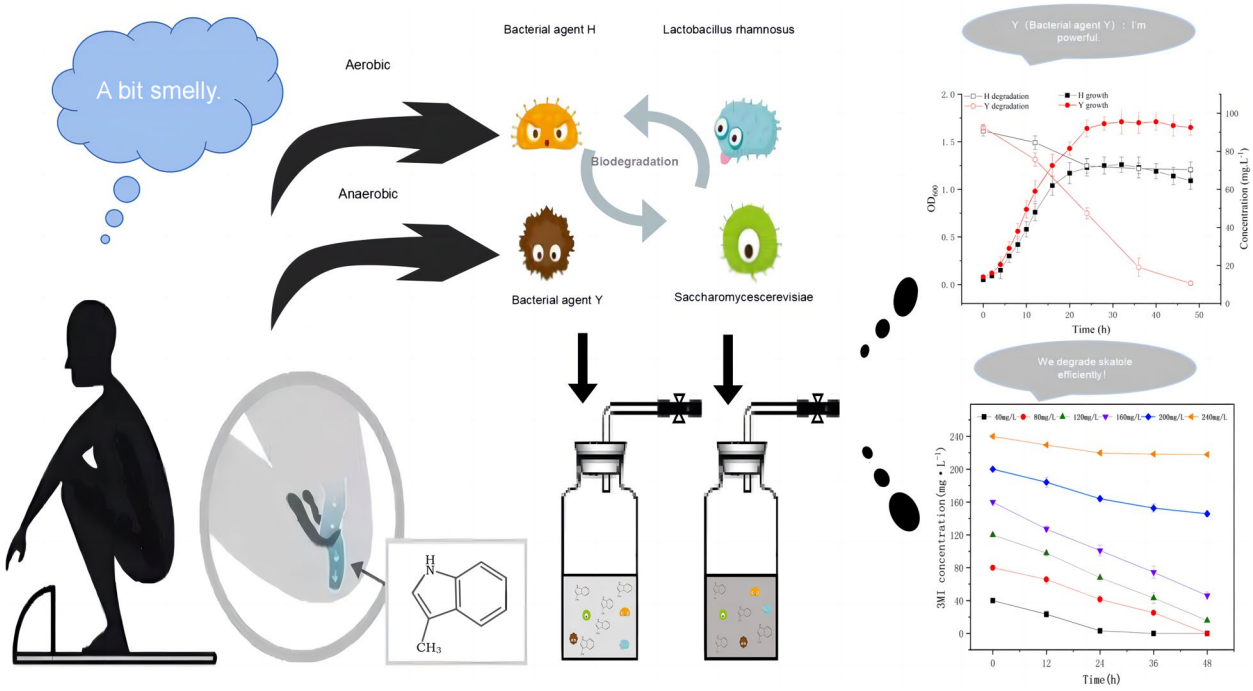
Results Microbial agents H and Y capable of utilizing skatole were screened out under aerobic and facultative anaerobic conditions, respectively. Within 48 h, the degradation rate of skatole by microbial agent Y was 88.52%. Following condition optimization, the optimum temperature for skatole degradation by microbial agent Y was 33 °C, and the optimum pH was 7. The main functional bacteria were *Acinetobacter xiamenensis*. The metabolites of skatole were determined by liquid chromatography-mass spectrometry (LC–MS), and six possible metabolites were found, including 3-aldehyde indole, 3-carboxyl indole, 1H-indole-2,3-dione, and 3-methyl indole pyruvate. The skatole-degrading bacteria *Lactobacillus rhamnosus* and *Saccharomyces cerevisiae* were then combined in proportion to form a composite deodorant. The 5-day degradation rate of skatole in the old manure group was 88.11%, while the 5-day degradation rate in the fresh manure group was 47.56%.

Conclusions The compound microbial agent developed in this study has a solid deodorizing effect, particularly in the control of the odor of obsolete feces. The use of composite microbial agents can efficiently degrade skatole in human feces, providing a theoretical foundation for the use of microbial remediation in the actual world.

Keywords Skatole, Human feces, Composite deodorant, Metabolic analysis, Microbial community

*Correspondence:
Jiangyu Ye
yejy@cqu.edu.cn
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Odor pollution is one of the world's seven major public hazards, as stated in the Basic Law of the Environment. Not only does odor pollution affect human emotions but also it has a negative impact on human health, causing abnormal shortness of breath, eye irritation, hoarseness, dry throat, toothache, and abnormal fatigue, fever or tremor, joint pain, and other symptoms (Aatamila et al. 2011). According to the Ministry of Ecology and Environment of China's website, a total of 531,176 public reports were received nationwide in 2019, with air pollution reports accounting for the most, accounting for 50.8% of all pollution; among air pollution, malodorous pollution reports had the most reports. The most common type of foul gas to which we humans are exposed in foul odor pollution is fecal foul gas.

Stool odor is associated with disrupting the balance of microorganisms (such as *bifidobacteria*) and harmful microorganisms (such as *Escherichia coli*) in the intestinal flora and the aging of the intestinal environment. Volatile fatty acids, sulfur compounds, indole, skatole, and amines are commonly blamed for the odor of human feces (Moore et al. 1987). Two-hundred ninety-seven volatile compounds have been detected in adult feces, according to reports (Garner et al. 2007). Studies employing headspace analysis and gas chromatography-mass spectrometry (GC-MS-O) with sniffing ports have indicated that specific phenols, indole, skatole, ammonia, and sulfur compounds play crucial roles in determining the characteristic odor of urine (Troccaz et al. 2013; Wagenstaller and Buettner 2013). Utilizing a similar approach, Lin et al. (2013) investigated the malodorous nature of pit toilets in India and Africa, identifying sulfur compounds, short-chain fatty acids, indole, skatole, and phenols as key components of human excrement odor.

Skatole, 3-methyl indole (3MI), is one of human feces' most visible and bothersome compounds (Schiffman et al. 2001; Liu et al. 2018). The olfactory threshold in air emissions is 0.00309 mg/m^3, which allows us to perceive chemicals at low concentrations. Skatole, in addition to its unpleasant pungent odor, is harmful to the ecosystem and human health. Skatole has been shown to cause acute bovine pulmonary edema, emphysema, lung disease, hemoglobinuria, and ruminant hemolysis (Carlson et al. 1972). Skatole's biotransformation products have been shown to covalently bind with lung proteins, posing potential harm to human lung cells (Weems et al. 2009). Furthermore, skatole exhibits bacteriostatic characteristics and exerts toxic effects on numerous microorganisms (Tittsler et al. 1935).

Skatole can be converted under conditions of anaerobic methanogenesis and sulfate reduction (Gu et al. 2002). Skatole removal efficiency has been relatively high in recent decades, so it has attracted much attention. The first studies were conducted in 1968, and the results demonstrated that indole-induced gram-positive cocci cells could oxidize skatole (Motoji and Hiroshi 1968). The *Pseudomonas* Gs strain isolated from mangrove sediments could mineralize skatole until 2006 (Yin et al. 2006). Subsequent studies found that *Lactobacillus* (Meng et al. 2013), *Rhodopseudomonas palustris* (Sharma et al. 2015), *Coprophilous* (Fukuoka et al. 2015), and *Acinetobacter* have skatole-removing properties (Tesso et al. 2019). However, due to the biological toxicity and recalcitrance of skatole, the number of reported microbial strains with skatole-degrading ability still needs to be improved, and the ability of each strain to degrade skatole varies greatly. So far, only one paper (Tesso et al. 2019) has mentioned using *Acinetobacter NTA1-2A* and *Acinetobacter TAT1-6A* to deal with fecal ozone in actual feces (chicken manure). However, there needs to be more relevant microbial community information.

Although reports on the degradation of skatole by single aerobic strains are common, there are few reports on the use of composite microbial agents for environmental remediation and skatole degradation. Compared with pure strains, the degradation ability of complex bacteria is more robust, practical, and efficient. In this study, a mixed microbial agent capable of degrading skatole was screened under aerobic and facultative anaerobic conditions, and its biodegradability under different conditions was evaluated. At the same time, the metabolites were analyzed, and the skatole-degrading bacteria were compounded with the existing NH_3 and H_2S deodorizing strains in our laboratory to make a composite deodorizer. The changes in odor substances (3-MI, NH_3, H_2S) and microbial community structure in feces were comprehensively analyzed to offer helpful bacterial resources for environmental odor pollution.

Materials and methods

Chemicals and mediums

High-performance liquid chromatography (HPLC) grade 3MI, methanol, and acetonitrile were purchased from Thermo Fisher Scientific. The reagents were biological grade and analytical reagent grade, which were purchased from Chongqing Chuandong Chemical Industry.

The minimal salt medium (MSM) (L^{-1}) (Tesso et al. 2019) is as follows: 1.0-g $K_2HPO_4{\cdot}3H_2O$, 0.20-g KH_2PO_4, 0.05-g $CaCl_2$, 0.5-g $MgCl_2$, 0.01-g $FeCl_2$, 1.0-g $(NH_4)_2SO_4$, 5.0-g NaCl, and 1.0-g yeast extract powder. The culture medium was autoclaved at 121 °C for 20 min, skatole was dissolved in methanol as a stock solution before use, and the stock solution was filtered through a 0.22-μm PTFE membrane and added to the culture medium.

Screening of skatole-degrading bacteria

Weigh two 2-g samples of septic tank feces, add them to 250-mL conical flasks containing 100 mL of modified MSM medium, and place the medium in a constant temperature shaker and an incubator. Perform aerobic and facultative anaerobic cultivation. The constant temperature shaker's parameters are as follows: 32 °C, 150 rpm; the constant temperature incubator's temperature is 32 °C. After 2 days, suck the culture from the 2-mL Erlenmeyer flask into two new MSM mediums with a pipette and continue culturing for 2 days. This process was repeated about 15 times, resulting in turbidity in the culture solution and significant degradation of the skatole.

Test of degradation effect of bacterial agents

Transfer the logarithmic growth culture solution to a 10-mL centrifuge tube, centrifuge at 5000 rpm for 5 min, then pour out the supernatant, wash the bacteria with sterile saline three times, and finally adjust the OD_{600} to 0.8–1.0 with saline. A total of 2 mL of the cleaned bacterial solution should be transferred to the new skatole-modified MSM medium. After thoroughly mixing, pipette 1 mL of culture solution into a 7-mL centrifuge tube as a 0-h sample, then inoculate the culture medium with bacteria H and bacteria Y, and place them in a constant temperature shaker and a constant temperature incubator for 12 h, sampling at 24 h, 36 h, and 48 h. All operations were performed in triplicate.

The parameters for the HPLC procedure entailed a C18 chromatographic column and a mobile phase composed of methanol and 1% acetic acid in a 1:1 volume ratio. The flow rate was set at 0.6 mL/min, with an injection volume of 20 μL, a detection wavelength of 60 nm, and a column temperature of 30 °C. An initial step involved the preparation of a standard curve correlating skatole concentration and peak area. Subsequently, the peak area ascertained from the actual sample was aligned with the standard curve to compute the sample's precise concentration.

Isolation and species identification of functional strains

The target microorganism was derived from the two mixed agents mentioned above. Sterilize the MSM culture medium, add the skatole reserve liquid to the ultra-clean worktable, thoroughly mix, and wait until the liquid surface bubbles disappear before pouring the plate and waiting to solidify, inverting it to standby. The culture medium was dipped in the inoculation ring, and the plate was lined using the three-zone method. Five plates were lined with aerobic and facultative anaerobic culture

mediums, respectively. In order to select single colonies with different shapes, the plate with good streaking effect and dispersed colonies was selected and then inoculated on the new plate, respectively. The plate inoculated with a single colony was streaked three times to obtain actual pure bacteria. After determining that the isolated pure bacteria could degrade skatole, each isolate was identified morphologically, physiologically, and biochemically.

Finally, the 16S rRNA sequence was extracted, amplified, and sequenced before being compared to known sequences in GenBank to determine the bacterial species.

Parameter optimization of degradation effect of skatole

It has been established that bacterial agent Y exhibits superior efficiency in skatole degradation. To further probe its potential, the impacts of varying parameters on skatole degradation were examined, including initial skatole concentrations (40, 80, 120, 200, 240 mg/L), temperatures (24, 27, 30, 33, 36 °C) and pH levels (4, 5, 6, 7, 8, 9) across each experimental group. Upon reaching the termination of the logarithmic growth phase, the bacterial agent was subjected to centrifugation at 8000 rpm for 5 min, with the supernatant subsequently discarded. The bacterial sample was then rinsed with sterile saline and centrifuged again. This operation was repeated thrice to eliminate any residual medium solution and undegraded skatole from the bacterial solution. Following the sterile saline wash, the optical density at 600 nm (OD_{600}) was adjusted to a range of 0.8–1.0.

The bacterial agent Y, cultivated to the termination of the logarithmic growth phase, was inoculated into a fresh MSM medium at a volume ratio of 2%. During the exploration of pH's impact on the skatole degradation efficiency by microbial agent Y, the initial pH of the culture medium was designated as 7. The initial skatole concentration was set at 80 mg/L, while the experimental temperatures were adjusted to 24, 27, 30, 33, and 36 °C.

Under the conditions described above, place the culture medium inoculated with bacterial agent Y in a constant temperature incubator for 48 h and collect 1 mL of culture solution samples in 7-mL centrifuge tubes at 0 h, 12 h, 24 h, 36 h, and 48 h to determine the skatole concentration. All operations were carried out in triplicate.

LC-MS determination of skatole metabolites

Skatole degradation metabolites were detected in a 250-mL conical flask with the microbial agent H serving as the control group HC and the experimental group HT and the microbial agent Y serving as the control group YC and the experimental group YT. A 100-mL MSM liquid medium was added to four groups. MSM medium was supplemented with 80 mg/L of skatole mother liquor in the HT and YT experimental groups. Skatole stock solution was not used in the HC and YC control groups. After three washes with sterile saline, 2 mL of bacterial solution was inoculated in both groups at the end of the logarithmic growth phase. The conical flasks of the blank group HC and the experimental group HT corresponding to the agent H were then cultured in a constant temperature shaker at 32 °C and 150 rpm. The conical flasks of the blank group YC and the experimental group YT corresponding to agent Y were cultured in a constant temperature incubator at 32 °C. After 48 h of culture, the bacterial solution was centrifuged for 10 min in a 2-mL sterile centrifuge tube at 12,000 rpm. The supernatant was filtered through a 0.22-μm filter membrane before being transferred to a new sterile centrifuge tube and analyzed using LC-MS/MS.

The separation procedure utilized an ACQUITY UPLC HSS T3 1.8 μm (2.1 × 150 mm) chromatographic column. The autoinjector was calibrated to a temperature of 8 °C, with an established flow rate of 0.25 mL/min and a column temperature of 40 °C. A 2-μL gradient injection was employed. The mobile phase comprised 0.1% formic acid in water (B2) and 0.1% formic acid in acetonitrile (A2) for elution. For negative ionization, a 5-mM ammonium formate in water (B1) and acetonitrile (A1) mixture was utilized. The eluent was subsequently subjected to analysis via an electrospray ionization (ESI) mass spectrometer in both positive and negative ion modes. Mass scanning was conducted within the range of 81 to 1000 m/z. Crucial operating parameters included a capillary temperature of 325 °C, a resolution of 70,000, and secondary fragmentation facilitated by higher-energy C-trap dissociation (HCD) at a collision voltage of 30 eV.

Deodorization experiment of compound deodorant on human feces

Human feces were used in the deodorization experiment with composite deodorant. The effects of deodorant on old feces and fresh feces were compared using old feces (group L) and fresh feces (group N). Old feces were collected from a community in Changshengqiao Town, Nanan District, Chongqing City; fresh feces were collected from healthy adult volunteers, aged 20–25 years old, male and female three, with no significant diseases and no antibiotics within 1 month. Fresh feces collected from volunteers were promptly placed in the laboratory's −18 °C refrigerator to prevent material deterioration and odor gas volatilization. The collected feces were thoroughly mixed and then grouped prior to the start of the experiment.

Upon the culmination of the logarithmic growth phase, the microbial agents H, Y, *Lactobacillus rhamnosus*, and *Saccharomyces cerevisiae* were integrated into a composite deodorizer in a 1:1:1:1 volume ratio, with the last

two identified as laboratory strains. Group L was supplemented with 200 mL of mature feces, whereas the control group LC was given 10% purified water, and the experimental group LT was enriched with 10% composite deodorizer. Group N was administered 200 mL of fresh feces, while the control group NC was supplemented with 10% purified water, and the experimental group NT was supplemented with 10% composite deodorizer. The deodorization experiment was conducted in twelve 1-L open glass bottles, with each group comprising of three replicates. Immediately upon the addition of these agents, each mixture was vigorously stirred with a glass rod to ensure uniform distribution of the bacterial agents.

The odor intensity, skatole concentration, NH_3 and H_2S concentration, and microbial diversity were measured during deodorization. The sampling intervals were set to 0 h, 6 h, 1 day, 2 days, 3 days, and 5 days.

Data processing and diversity analysis

The V3–V4 region of the 16S rRNA gene was sequenced in the aforementioned aged and fresh fecal samples. Sequences with a similarity exceeding 97% were grouped into a single operational taxonomic unit (OTU), with total OTUs ranging between 26,297 and 49,996. To mitigate errors attributable to inter-sample variability, we employed the minimum OTU count for normalization, ensuring comparative analysis at the same OTU level across all samples.

The raw data from high-throughput sequencing were processed on the Meiji I-Sanger cloud platform https://cloud.majorbio.com. The Alpha-diversity index Chao and Simpson index were calculated using mothur (Schloss et al. 2009) software http://www.mothur.org/wiki/Calculators/, and the alpha-diversity difference between groups was analyzed using the Wilcoxon rank-sum test. The PCoA (principal coordinate analysis) algorithm was used to test the similarity of the microbial communities between the samples, and the PERMANOVA nonparametric test was used to determine whether the difference in microbial community structure between the sample groups was significant.

Results and discussion

Screening and identification of skatole-degrading bacteria

Utilizing septic tank feces as source material, two types of mixed bacterial cultures capable of fecal odor degradation were identified. The bacterial culture propagated in an aerobic incubator was designated as H, while the culture developed in a facultative anaerobic incubator was labeled Y. The individual bacterial strains isolated from agents H and Y demonstrated limited skatole degradation capacities, which could be attributed to their possible auxotrophic nature, necessitating coexistence with other microbial flora for normal metabolic activity. Hence, the community structure of bacterial agents H and Y, as depicted in Fig. 1a, was analyzed to ascertain the diversity and abundance of species within bacterial agents H and Y.

The skatole degradation ability of microbial agent H and microbial agent Y was determined in a conical flask. The results are depicted in Fig. 1b; skatole degradation rates by agent H and agent Y within 48 h were 22.39% and 88.52%, respectively, when the initial concentration of skatole was around 80 mg/L. When the strain reached the logarithmic phase, the skatole degradation rate increased, indicating that skatole degradation was positively correlated with strain growth to some extent. Based on the growth rate of bacteria and the content of bacteria in the culture medium, it was determined that the bacterial agent cultured for 28 h would be used for sampling and determining various experimental indicators in subsequent experiments.

Seven single strains were isolated from bacterial agent H, and five single strains were isolated from bacterial agent Y by plate streaking method to further determine the functional strains in the two bacterial agents, as shown in Fig. 1c.

When the degradation effect of a single isolated strain on skatole was tested, strain Ya isolated from strain Y was able to completely degrade skatole with an initial concentration of 80 mg/L in 72 h. Yin et al. (2006) collected mangrove sediments in Hong Kong for enrichment culture, but no effective skatole-degrading single bacteria were isolated. The enriched flora could completely degrade 2.0-mM skatole in 3 days, but a single bacteria isolated from the enriched flora could do so in 24 days.

When the 16S sequencing results of strain Ya were compared to the NCBI database, it was discovered that Ya was *Acinetobacter* with the highest homology with *Acinetobacter xiamenensis*, reaching 98.29%, as shown in Fig. 1d. This is consistent with the findings of Tesso et al. (2019).

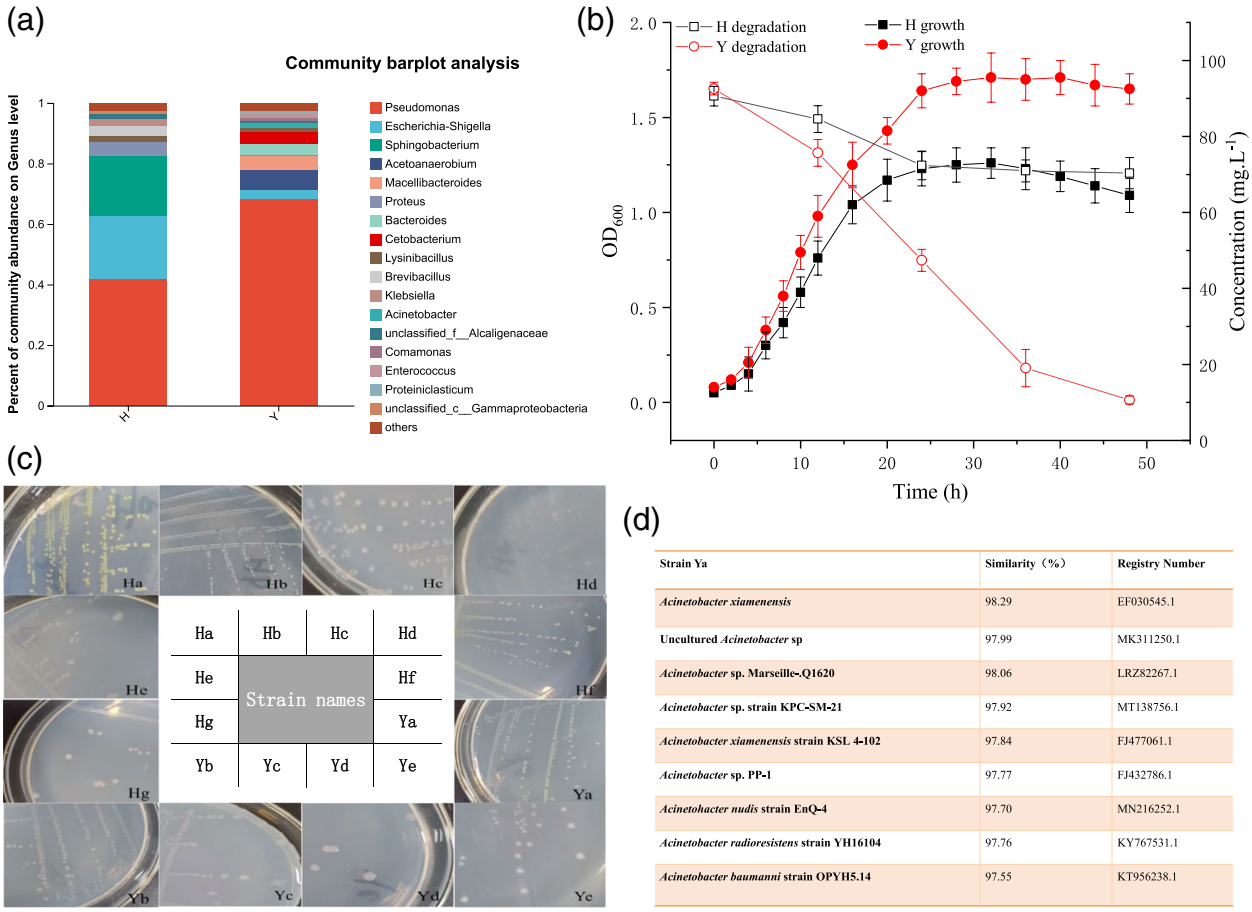
Optimization of bacteria degradation conditions

Temperature

As shown in Fig. 2a, the degradation rate of skatole by bacterial agent Y gradually increased as the temperature rose from 24 to 33 °C. At 33 °C, the maximum degradation rate of bacterial agent Y was 95.36%.

pH value

The pH of the culture medium will affect the growth of the strain and the degradation ability of skatole to a certain extent. According to Fig. 2b, when pH was 7, the degradation rate was as high as 95.77%. The degradation rate dropped to less than 40% when the pH value was



Strain Ya	Similarity (%)	Registry Number
Acinetobacter xiamenensis	98.29	EF030545.1
Uncultured *Acinetobacter* sp	97.99	MK311250.1
Acinetobacter sp. Marseille-Q1620	98.06	LRZ82267.1
Acinetobacter sp. strain KPC-SM-21	97.92	MT138756.1
Acinetobacter xiamenensis strain KSL 4-102	97.84	FJ477061.1
Acinetobacter sp. PP-1	97.77	FJ432786.1
Acinetobacter nudis strain EnQ-4	97.70	MN216252.1
Acinetobacter radioresistens strain YH16104	97.76	KY767531.1
Acinetobacter baumanni strain OPYH5.14	97.55	KT956238.1

Fig. 1 Screening and identification of skatole-degrading bacteria from human feces. **a** The community composition of the bacterial liquid at the genus level. **b** Growth and skatole-degrading curves of bacteria H and Y. **c** Colonies isolated from bacteria agent H and Y. **d** Comparison result of strain Ya

between 8 and 9. However, an excessively low pH (pH 4) served to inhibit the degradation activity of the microbial agent.

Initial concentration

Skatole exhibits toxicity towards microorganisms, with high concentrations inhibiting their activity. Figure 2c illustrates the degradation of skatole by bacterial agent Y under optimal temperature and pH conditions. Upon optimization of these conditions, the degradation rate of skatole by bacterial agent Y showed notable improvement. The agent retained a high skatole degradation capability (>72.87%) when its concentration was below 160 mg/L. However, a sharp decline in the degradation rate was observed when skatole concentrations reached 200 mg/L.

In the study conducted by Yin and Gu (2006), the degradation of skatole was observed to be notably slow when its concentration was increased to 3.5 mM. Since its inherent toxicity, this elevated concentration of skatole resulted in the inhibition of the bacterial strain responsible for its degradation. Facilitated by methanogens derived from wetland soil, 0.3 mM of 3-methylindole (3MI) underwent a transformation into 3-methylhydroxyindole within 100 h (Gu and Berry 1992). Kohda et al. (1997) successfully isolated three skatole-degrading strains of *Clostridium* from composted pig and chicken manure, demonstrating their capacity to degrade skatole concentrations ranging from 100 to 300 mg/L within 4 weeks. Compared to the strains previously mentioned, the bacterial agent Y in this study (pH 7.0, 150 r/min) exhibited superior degradation ability, completely degrading approximately 80 mg/L of skatole within 48 h.

Analysis of skatole metabolites

The supernatant obtained post-centrifugation of the culture broth was subjected to non-targeted liquid chromatography-mass spectrometry (LC-MS) to elucidate the metabolites resulting from skatole degradation by bacterial agent Y. The derived data was subsequently utilized

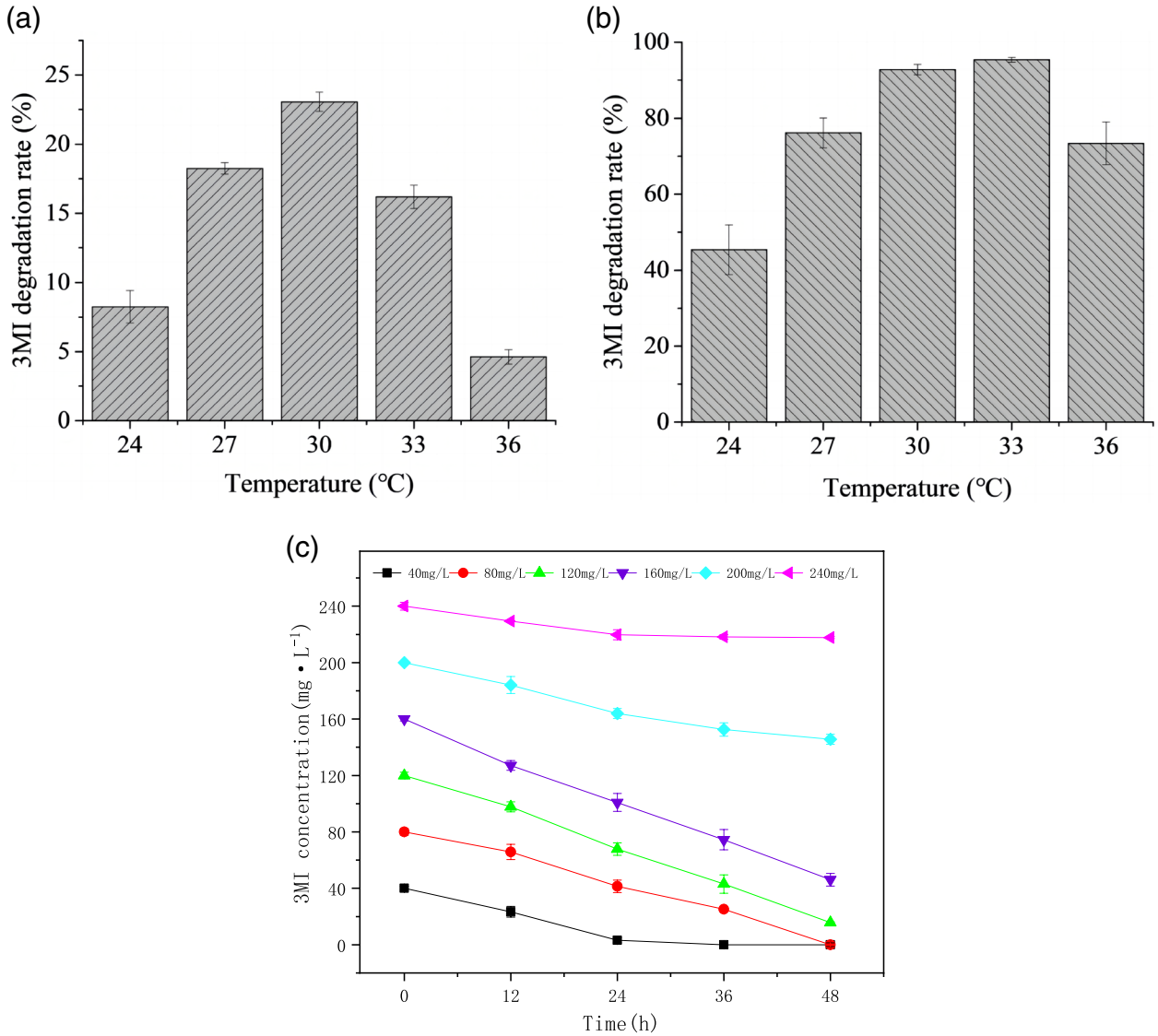


Fig. 2 Degradation effect of bacterial agent Y on skatole under different conditions. **a** When the temperature was 33 °C, the initial concentration of skatole was 80 mg/L, and the effect of initial pH on the degradation rate of skatole was studied. **b** When the initial pH of the medium was 7 and the initial concentration of skatole was 80 mg/L, the effect of temperature on the degradation rate of skatole was studied. **c** Under the conditions of 33 °C, pH 7, and 150 r/min, skatole degradation curves of bacterial agent Y with different initial concentrations were obtained

for bioinformatics analysis. Given the plethora of metabolites and the relatively low concentration of skatole and its metabolites of interest, direct screening of pertinent metabolite data was challenging. However, it is imperative to note that distinct metabolites play specific roles in biological systems. To elucidate these roles, we annotated all identified metabolite data with the KEGG database br08001, as presented in Fig. 3.

The carbohydrate metabolism pathway was observed to be diminished in both the HT and YT groups. This could be due to the potential antibacterial and toxic effects of skatole present in the experimental group, which may have inhibited their carbohydrate metabolism. Conversely, the augmentation of the lipid metabolism pathway was consistent with the decrease in skatole concentration throughout the experiment. Owing to skatole's lipophilic nature, it tends to accumulate within cellular lipids. Some bacteria in the experimental group may utilize these skatole-lipid conjugates as energy sources for their growth. The subsequent rise in skatole-related metabolites suggests that low concentrations of skatole may accumulate in lipids over time.

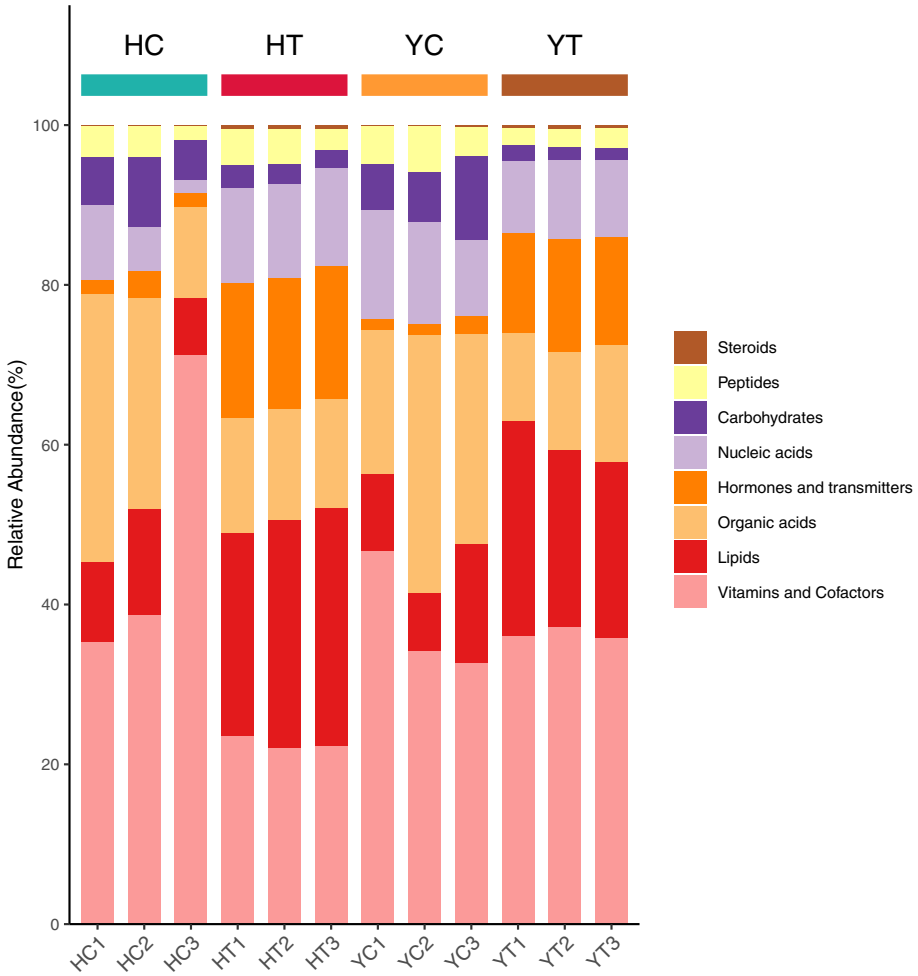


Fig. 3 The role-playing bar chart of metabolite biology

The application of partial least squares-discriminant analysis (PLS-DA) facilitated the identification of different metabolites between groups. PLS-DA plots pertaining to bacterial agents H (HC and HT) and Y (YC and YT) are illustrated in Fig. 4a and b. Metabolites within the highlighted yellow region of the figure have a variable importance in projection (VIP) value exceeding 1 and a *p*-value below 0.05, signifying considerable variance among groups. It is noteworthy that these metabolic discrepancies could be induced by the introduction of skatole into the medium.

Drawing from the differential metabolites presented in Fig. 4, a selection of 36 metabolites were further isolated based on their compound structural formulas. A corresponding heatmap is illustrated in Fig. 5. Cluster analysis reveals that the metabolites of control groups HC and YC exhibit similarity, as do the metabolites of experimental groups HT and YT. Procollagen 5-hydroxy-L-lysine, quinoline, 3-amino-4-hydroxybenzoate, and 2-phenylethanol are among the metabolites found in high concentrations in HC and YC.

According to the structural formula and chemical properties of the metabolites, 6 products that may be closely related to skatole metabolism were screened from the different metabolites, as shown in Fig. 6, namely: 3-aldehyde indole and 3-carboxyindole, 3-methylhydroxyindole, 1H-indole-2,3-dione, 3-indoleacetamide, 2-amino-3-methoxybenzoic acid,3-methylindolepyruvate, and pindolol.

As evidenced in the existing literature, the 3-methylhydroxyindole identified in this study is a well-established metabolite. The enriched wetland soil methanogens (Gu and Berry 1992) were metabolized by converting 3-methylindole to 3-methyloxindole. Gu et al. (2002) also proposed that marine anaerobic microorganisms degrade 3MI via the 3-methylhydroxyindole and α-methyl-2-aminobenzencacetic acid pathways. The 3-carboxyindole (indole-3-carboxylic acid) detected in this experiment

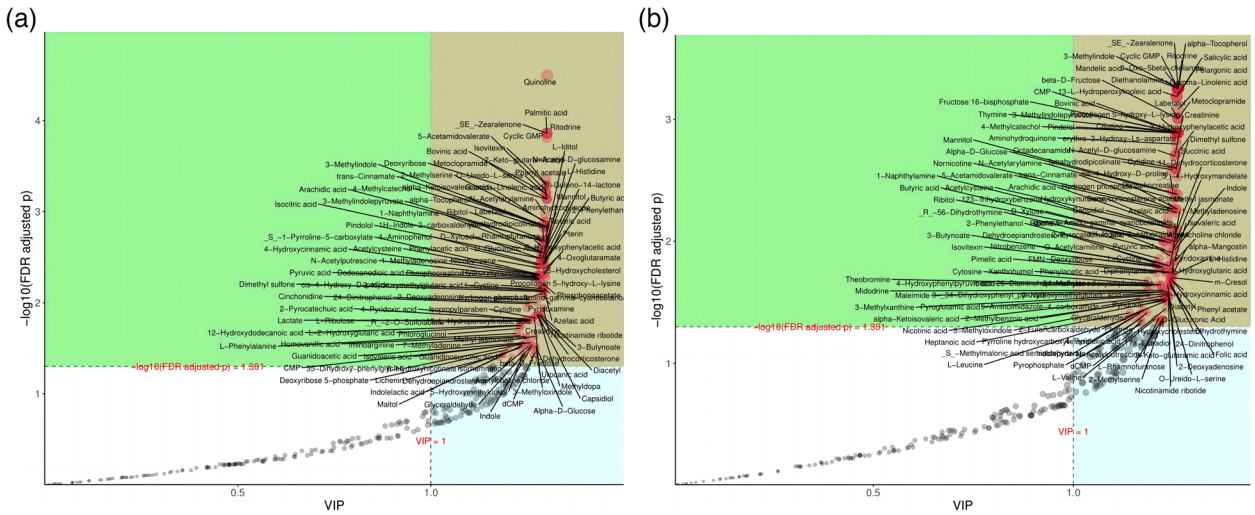


Fig. 4 PLS-DA metabolite importance map. **a** Bacteria H. **b** Bacteria Y

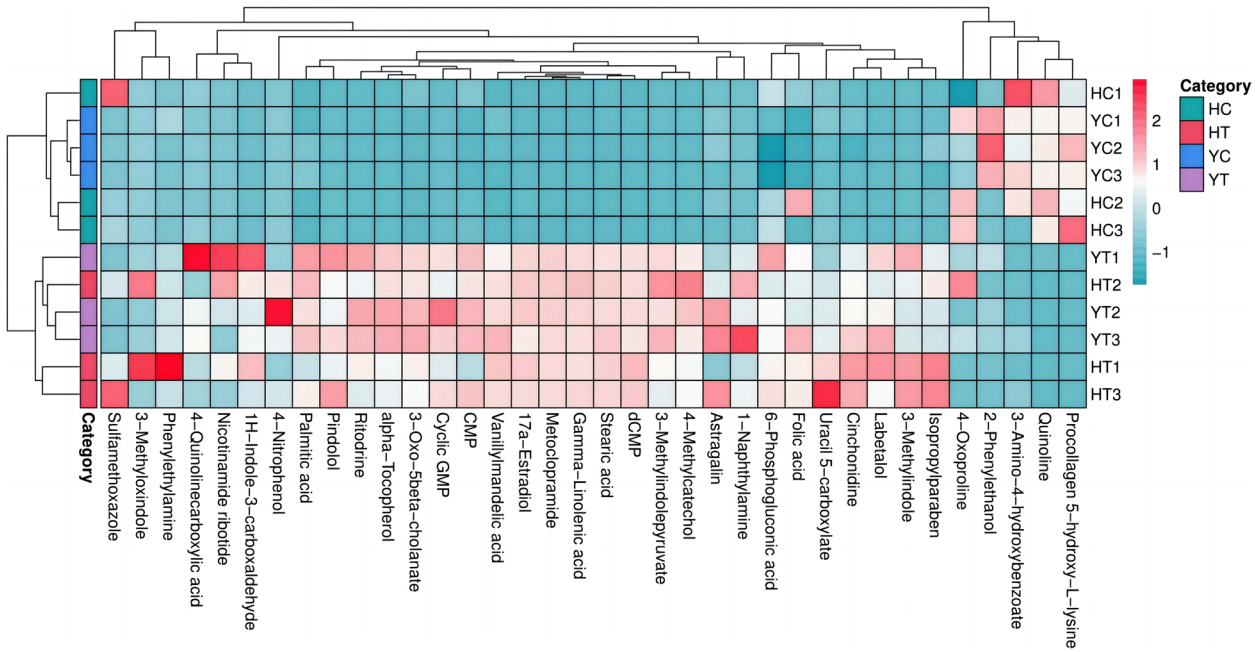


Fig. 5 Heatmap of metabolites (HC, bacterial agent H control group; HT, bacterial agent H experimental group; YC, bacterial agent Y control group; YT, bacterial agent Y experimental group)

has previously been reported. *Pseudomonas aeruginosa* (Yin and Gu 2006) degraded 3-methylindole to produce two metabolites, indoline-3-carboxylic acid, and indoline-3-ol. The biotransformation of the carbocyclic aromatic ring of 3-methylindole into a monocyclic pyrrole carboxylic acid has also been confirmed as the biodegradation of co-metabolized 3-methylindole (Fukuoka et al. 2015). Furthermore, this experiment detected the presence of 1H-Indole-2,3-dione(isatin), which can be obtained by oxidizing 3-methylhydroxyindole further. Isatin was reported to be a common metabolite of indole (Yang et al. 2018); it is conceivable that isatin could be the product of 3-methylindole degradation to indole, followed by subsequent metabolism.

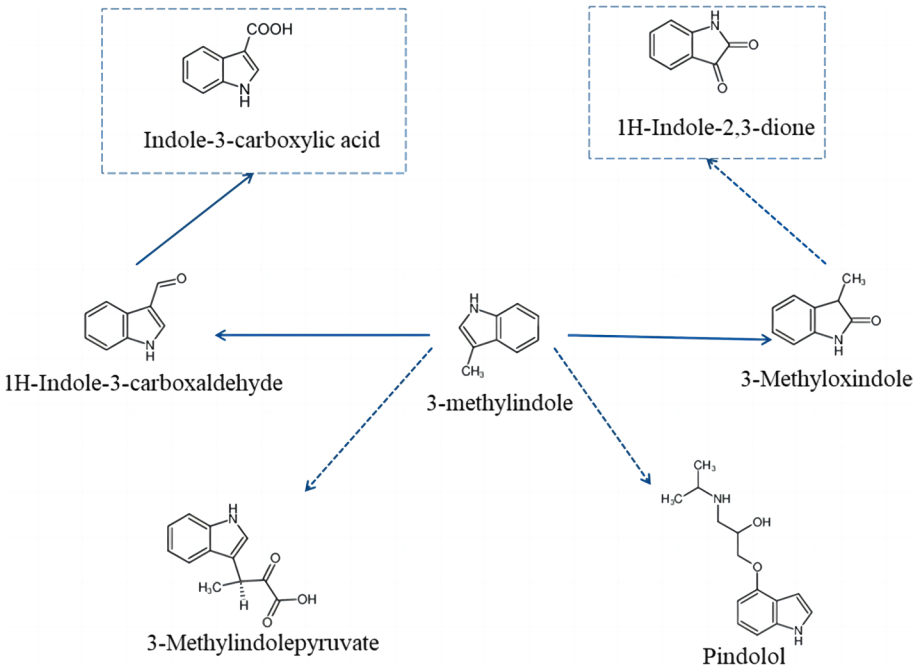


Fig. 6 Chemical structure of possible metabolites of 3MI

Changes in the concentration of malodorous substances

The variation in skatole content throughout the experiment was analyzed via liquid chromatography, with the results presented in Fig. 7a. The LT group, utilizing stale feces, demonstrated a rapid initial skatole degradation within the first 24 h, which subsequently slowed. By the 120-h mark, the degradation rate had peaked at 88.11%. Conversely, in the NT group using fresh feces, the skatole concentration decreased from an initial 0.82 to 0.43 mg/L, achieving a degradation rate of 47.56%.

NH_3 and H_2S have been reported in numerous studies as traditional odor compounds (Liu et al. 2012). Figure 7b and c showed the changes in NH_3 and H_2S during the experiment. The LT group (old manure) and NT group (fresh manure) of the experimental treatment exhibited the lowest concentrations within 24 h. This outcome can be attributed to the logarithmic growth phase of the introduced deodorant, enabling efficient utilization of fecal nutrients for reproduction and simultaneous rapid removal of NH_3 and H_2S. Although the concentrations of LC and NC in the control group showed some reduction within the initial 24 h, the impact was considerably less pronounced compared to the experimental groups. Notably, the NH_3 content in the LT group of aged feces increased at a faster rate, while the H_2S content in the NT group of fresh feces increased more rapidly. These findings suggest that the added microbial agent influenced the original colony composition of the ecosystem through interference, and the ecosystem exhibited resistance to the introduced microbial agent (Shade et al. 2012). This resistance may also account for the unsuccessful colonization observed with certain strains during the process of bioaugmentation.

Analysis of microbial community structure

The V3–V4 region of the 16S rRNA gene from the aforementioned stale fecal samples and fresh samples was sequenced, resulting in a total of 1,526,798 unique sequences. The rarefaction curve (Fig. S1) demonstrated that the number of sequences had reached saturation, ensuring that the subsequent analysis accurately reflected the microbial composition of the samples. Figure S2 illustrates the α-diversity of each group. The Shannon indexes for groups L and N were approximately 5.00 and 3.46, respectively. Throughout the process, both the Chao index and Ace index in group L exceeded those in group N, indicating a richer community in group L. Overall, group L exhibited a higher α-diversity index compared to group N, which also corresponded to a higher skatole-scavenging performance, suggesting a positive correlation trend.

In terms of β-diversity, PCoA based on Bray-Curtis distance matrix was used to reveal community differences. The results showed that the microbial communities of the four groups were separated from the original flora after 5 days of experiment, and the microbial communities of group L and group N were significantly different during the experiment (Fig. 8).

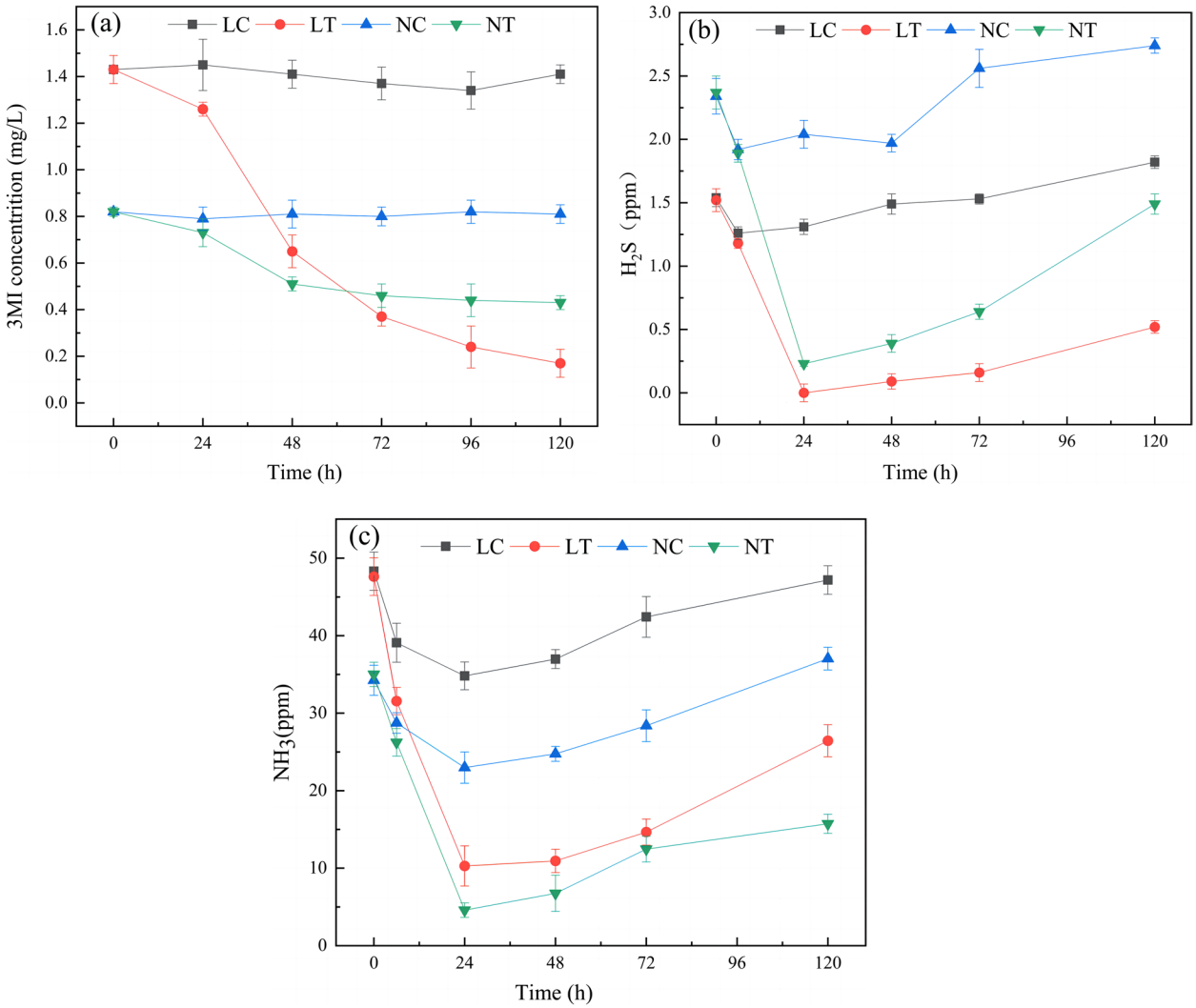


Fig. 7 Changes in the concentration of odorous substances during the deodorization process. **a** Skatole. **b** H_2S. **c** NH_3

The microbial composition at the genus level is depicted in Fig. S3. *Firmicutes*, as the dominant phylum, is observed in both group L and group N. However, in the final stage, its presence decreases from 54.45 to 31.63% in group L and from 85.89 to 65.29% in group N. Meanwhile, the concentration of *Actinobacteriota* in group L diminishes from 14.43 to 6.17%, whereas in group N, it escalates from 12.30 to 23.66%.

The microbial community structure at the genus level is illustrated in Fig. 9. A comprehensive analysis of this microenvironment reveals substantial alterations in the distribution of functional bacteria. In the sterilized LC group, the *Lactobacillus* count is virtually non-existent, as illustrated in Fig. 9a. In contrast, the bacterially inoculated LT group demonstrates a prominent surge in *Lactobacillus* count at the 24-h mark. Interestingly, this proliferation corresponds with the lowest recorded concentrations of NH_3 and H_2S in the stale fecal samples. These findings emphasize the integral role of lactic acid bacteria in mitigating these substances, further accentuating the potential influence of microbial communities on waste decomposition processes. A strain of *Lactobacillus* KJ-10311 isolated by Kim and Park (2006) can effectively remove volatile fatty acids and NH_3 in pig manure. Moreover, a study by Meng et al. (2013) demonstrated that *Lactobacillus brevis* can achieve a 65.35% removal rate of 1.0 μg/ml 3MI within 120 h.

On day 5, the *Acinetobacter* content was merely 0.64% in the LC group yet escalated to 3.43% in the LT group. As a functional strain in agent Y, the rise in *Acinetobacter* suggests its potential role in skatole degradation, possibly utilizing skatole for metabolic sustenance and growth.

Within the fresh feces group, observed shifts in the prevalence of dominant and functional bacteria are

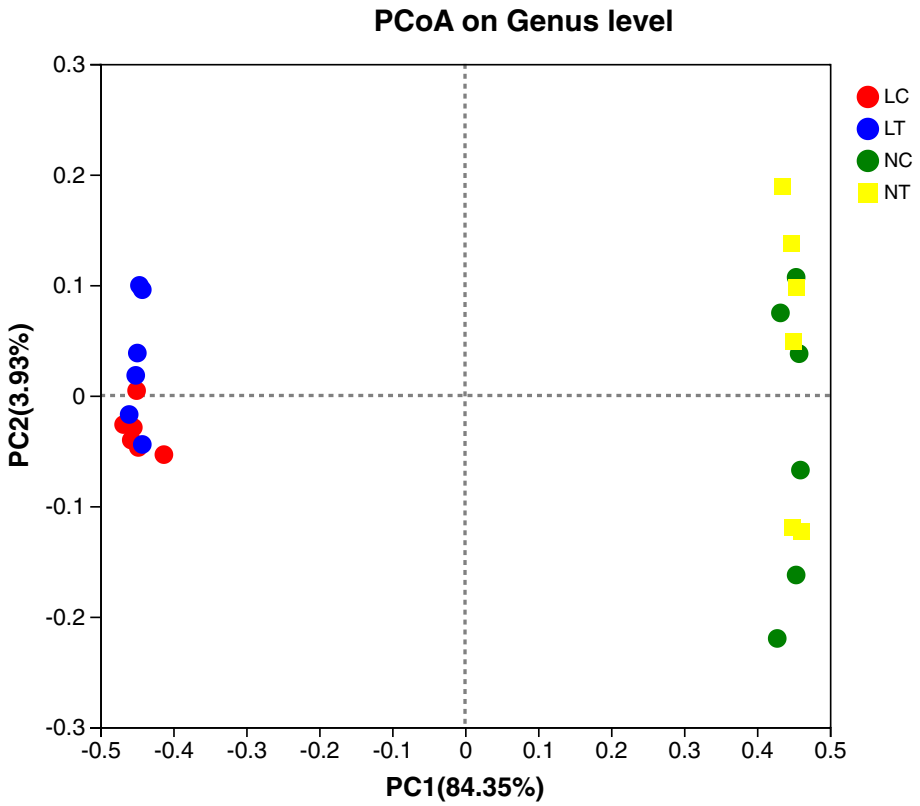


Fig. 8 PCoA plot of the bacterial communities

detailed in Fig. 9b. At the initiation of our experimental process, *Bifidobacterium* represented the primary bacterial genus in both the sterilized NC group and the bacterially inoculated NT group, each boasting an initial abundance of 10.27%. By the conclusion of the experiment, there was a notable increase in the abundance of *Bifidobacterium* to 13.67% in the NC group and 17.14% in the NT group, respectively.

Eubacterium_hallii followed, demonstrating a consistently increasing trend. Research has indicated that *Eubacterium_hallii* is a beneficial bacterium with the capability to degrade monosaccharides, yet it lacks the ability to degrade complex oligosaccharides and polysaccharides (Vera et al. 2018). However, *Bifidobacterium* has been found to facilitate polysaccharide degradation; thus, the proliferation of *Bifidobacterium* also supports the subsequent proliferation of *Eubacterium_hallii*.

As a constituent of the deodorant, the content of *Lactobacillus* exhibited a significant increase in both the experimental and control groups. *Lactobacillus* content reached its peak at the 24-h mark, which aligned with the previously identified *Lactobacillus* strain in the medium entering a stable period and attaining maximum growth at 20 h. Contrary to the stale feces group, where *Lactobacillus* content initially increased and then decreased, the *Lactobacillus* content in fresh feces surged to 10.09% on the 5th day, thereby emerging as the dominant flora. The addition of *Lactobacillus* to fecal sludge, as reported by Odey et al. (2018), can inhibit the growth of fecal coliforms, thereby reducing the prevalence of pathogenic bacteria.

Conclusion

In this study, skatole-utilizing bacteria H and Y were acclimated under aerobic and facultative anaerobic conditions. When the initial skatole concentration in the mixed bacterial degradation system was below 160 mg/L, Y achieved a skatole degradation rate exceeding 72% within 48 h. LC–MS analysis revealed similar skatole degradation products for microbial agents H and Y, identifying 3-formylindole, 3-carboxyindole, 3-methylhydroxyindole, 1H-indole-2,3-dione, 3-methylindole pyruvate, and indolylol as skatole metabolites. A composite deodorant formulated from these two microbial agents effectively deodorized both fresh and stale feces and efficiently removed skatole, NH_3, and H_2S. High-throughput sequencing results revealed marked diversity and community structure disparities between groups L

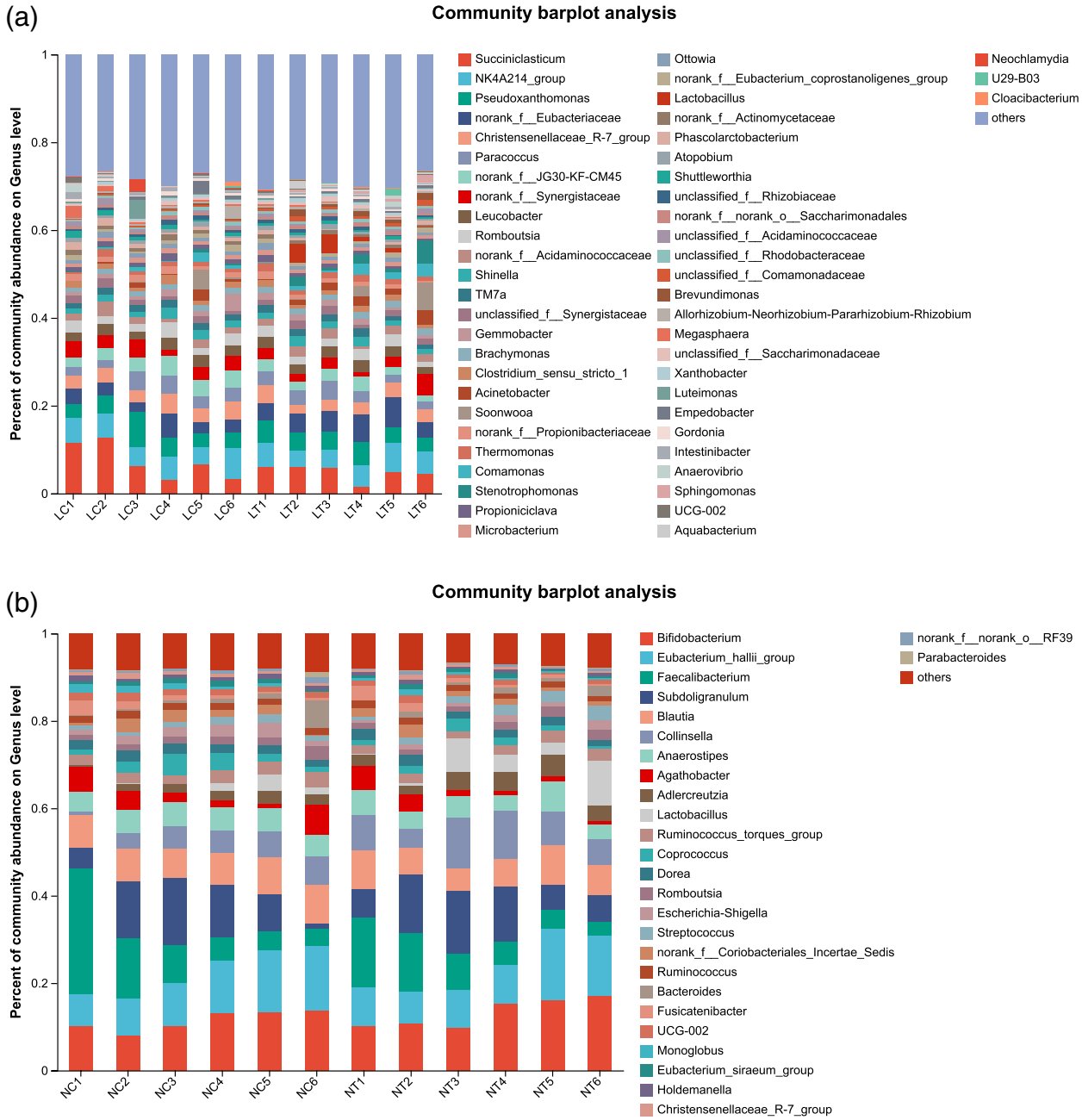


Fig. 9 Level of microbial community analysis on genus level (**a**). Old feces group L. **b** Fresh feces group N

and N, with a significant increase in *Acinetobacter* content in group L, while *Lactobacillus* dominated in group N. The composite microbial agent exhibited strong deodorizing efficacy on stale feces, potentially serving as an effective deodorant for septic tanks. The frequency of deodorizing agent addition, however, should be tailored to specific circumstances to maintain long-term deodorizing effectiveness.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-023-01722-x.

Additional file 1: Fig. S1. Rarefaction curve. **Fig. S2.** Species diversity analysis. **Fig. S3.** Circos plot of the bacterial communities (a) Old feces group L, (b) Fresh feces group N.

Additional file 2.

Acknowledgements
The study was supported by the Science and Technology Planning Project of Chongqing Ecology and Environment Bureau, China (grant number 2019-125). We also thank Prof. Jiangyu Ye for technical support.

Authors' contributions
JY, conceptualization, funding acquisition, supervision, and writing — review and editing. QF, data curation, investigation, methodology, software, visualization, and writing — original draft. The author(s) read and approved the final manuscript.

Funding
This work was supported by the Science and Technology Planning Project of Chongqing Ecology and Environment Bureau, China (grant number 2019-125).

Availability of data and materials
In Qian Fu and Jiang-yu Ye (2023), the original data and material for "Screening of Fecal Odor Degrading Bacteria and Control of Human Fecal Odor by Compound Bacteria" are recorded in the "data record sheet" and "supplemental material," which are attached to this article.

Declarations

Ethics approval and consent to participate
The study did not violate ethics, and all participants agreed to publish the paper.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]College of Environment and Ecology, Chongqing University, Chongqing 400045, China. [2]Key Laboratory of Three Gorges Reservoir Region's Eco-Environment, Ministry of Education, Chongqing University, Chongqing 400045, China.

Received: 5 February 2023 Accepted: 22 May 2023
Published online: 24 June 2023

References

Aatamila M et al (2011) Odour annoyance and physical symptoms among residents living near waste treatment centres. Environ Res 111:164–170
Carlson JR, Yokoyama MT, Dickinson EO (1972) Induction of pulmonary edema and emphysema in cattle and goats with 3-methylindole. Science 176:298–299
Fukuoka K, Ozeki Y, Kanaly RA (2015) Aerobic biotransformation of 3-methylindole to ring cleavage products by Cupriavidus sp. strain KK10. Biodegradation 26:359–373
Garner CE et al (2007) Volatile organic compounds from feces and their potential for diagnosis of gastrointestinal disease. FASEB J 21:1675–1688
Gu JD, Berry DF (1992) Metabolism of 3-methylindole by a methanogenic consortium. Appl Environ Microbiol 58:2667–2669
Gu JD, Fan YZ, Shi HC (2002) Relationship between structures of substituted indolic compounds and their degradation by marine anaerobic microorganisms. Mar Pollut Bull 45:379–384
Kim JD, Park KM (2006) Effectiveness of Lactobacillus plantarum strain KJ-10311 to remove characteristic malodorous gases in piggery slurry. Asian-Aust J Anim Sci 19:144–152
Kohda C, Ando T, Nakai Y (1997) Isolation and characterization of anaerobic indole- and skatole-degrading bacteria from composting animal wastes. Appl Microbiol Mol Cell Biosci Res Found 43:249–255
Lin JM et al (2013) Qualitative and quantitative analysis of volatile constituents from latrines. Environ Sci Technol 47:7876–7882
Liu XZ, Dong ZG, Li GB (2012) Technical research on the treatment of hydrogen sulfide and ammonia using bio-deodorization technology. China Sci Technol Overview 20:51
Liu DZ et al (2018) Indoleacetate decarboxylase is a glycyl radical enzyme catalysing the formation of malodorant skatole. Nat Commun 9:4224
Meng X, He ZF, Li HJ, Zhao X (2013) Removal of 3-methylindole by lactic acid bacteria in vitro. Exp Ther Med 6:983–988
Moore JG, Jessop LD, Osborne DN (1987) Gas-chromatographic and mass-spectrometric analysis of the odor of human feces. Gastroenterology 93:1321–1329
Motoji F, Hiroshi W (1968) The bacterial oxidation of indole. Elsevier 158:70–78
Odey EA, Li Z, Zhou X et al (2018) Locally produced lactic acid bacteria for pathogen inactivation and odor control in fecal sludge. J Clean Prod 184:798–805
Schiffman SS, Bennett JL, Raymer JH (2001) Quantification of odors and odorants from swine operations in North Carolina. Agric For Meteorol 108:213–240
Schloss PD, Westcott SL, Ryabin T et al (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541
Shade A, Peter H, Allison SD et al (2012) Fundamentals of microbial community resistance and resilience. Front Microbiol 3:417
Sharma N, Doerner KC, Alok PC, Choudhary M (2015) Skatole remediation potential of Rhodopseudomonas palustris WKU-KDNS3 isolated from an animal waste lagoon. Lett Appl Microbiol 60:298–306
Tesso TA, Zheng AJ, Cai HY, Liu GH (2019) Isolation and characterization of two Acinetobacter species able to degrade 3-methylindole. PLoS One 14:e0211275
Tittsler RP, Sandholzer LA, Callahan ET (1935) The bacteriostatic action of skatole on gram-negative enteric bacilli. J Infect Dis 57:57–60
Troccaz M, Niclass Y, Anziani P, Starkenmann C (2013) The influence of thermal reaction and microbial transformation on the odour of human urine. Flavour Fragr J 28:200–211
Vera B, Christophe L, ClarissaL S (2018) Mucin cross-feeding of infant bifidobacteria and Eubacterium hallii. Microb Ecol 75:1
Wagenstaller M, Buettner A (2013) Quantitative determination of common urinary odorants and their glucuronide conjugates in human urine. Metabolites 3:637–657
Weems JM et al (2009) 3-Methylindole is mutagenic and a possible pulmonary carcinogen. Toxicol Sci 112:59–67
Yang BY, Lin YX, Dai CX et al (2018) Characterization of an indole-degrading bacterium Alcaligenes sp.YBY. Environ Sci Technol 41:1–6
Yin B, Gu JD (2006) Aerobic degradation of 3-methylindole by Pseudomonas aeruginosa Gs isolated from mangrove sediment. Hum Ecol Risk Assess Int J 12:248–258
Yin B, Huang LM, Gu JD (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

Publisher's Note