Biodegradation and metabolic pathway of sulfamethoxazole by *Sphingobacterium mizutaii*

Jinlong Song¹,5, Guijie Hao²,5, Lu Liu¹, Hongyu Zhang¹, Dongxue Zhao¹,³, Xingyang Li¹,³, Zhen Yang¹, Jinhua Xu¹, Zhiyong Ruan⁴, & Yingchun Mu¹

Sulfamethoxazole (SMX) is the most commonly used antibiotic in worldwide for inhibiting aquatic animal diseases. However, the residues of SMX are difficult to eliminate and may enter the food chain, leading to considerable threats on human health. The bacterial strain *Sphingobacterium mizutaii* LLE5 was isolated from activated sludge. This strain could utilize SMX as its sole carbon source and degrade it efficiently. Under optimal degradation conditions (30.8 °C, pH 7.2, and inoculum amount of 3.5 × 10⁷ cfu/mL), *S. mizutaii* LLE5 could degrade 93.87% of 50 mg/L SMX within 7 days. Four intermediate products from the degradation of SMX were identified and a possible degradation pathway based on these findings was proposed. Furthermore, *S. mizutaii* LLE5 could also degrade other sulfonamides. This study is the first report on (1) degradation of SMX and other sulfonamides by *S. mizutaii*, (2) optimization of biodegradation conditions via response surface methodology, and (3) identification of sulfanilamide, 4-aminothiophenol, 5-amino-3-methylisoxazole, and aniline as metabolites in the degradation pathway of SMX in a microorganism. This strain might be useful for the bioremediation of SMX-contaminated environment.

The amount of aquaculture in China ranks first in the world all year round. The huge output mainly depends on high-density aquaculture¹. This type of aquaculture leads to the risk of outbreaks of aquatic animal diseases. Sulfonamides are synthetic drugs used as anti-microbial, anti-diabetic, diuretic, anticonvulsant, and herbicidal agents. Among which, sulfamethoxazole (SMX) is one of the most prescribed antibiotics worldwide, can kill aquatic pathogens and can effectively inhibit aquatic animal diseases²–⁴. However, SMX cannot be completely utilized and metabolized by aquatic animals because approximately 50% are excreted to the environment without modification. Consequently, SMX has become a widely distributed pollutant in aquatic and domestic waste waters⁵. Numerous studies have indicated that SMX has adverse ecological effects and may threaten human health after long-term exposure. The World Health Organization has classified SMX as a Category 3 carcinogen in 2017⁶. However, SMX has been proven to be difficult to eliminate by conventional water treatment processes⁷. The residues of SMX enter the food chain, leading to considerable threats to human health. Therefore, an efficient and reliable degradation method for removing SMX residue from the water environment must be developed⁸.

SMX removal is mainly by physical, chemical, and microbial methods⁹. The physical and chemical methods mainly include anion exchange, nano-filtration, struvite precipitation, and electrodialysis¹°. However, for technical and economic reasons, physical and chemical techniques may not be feasible¹¹. Microbial biodegradation has advantages in terms of its eco-friendliness, low cost, and scope of implementation and has been proven as an effective method for the remediation of SMX residues in aquaculture waters¹². To date, more than 20 strains of SMX-degrading strains have been isolated from different environments, and all of them exhibit SMX-degrading ability under laboratory conditions. Liang et al. isolated *Achromobacter* sp. JJ9, which can use SMX as the sole nitrogen source for growth. The degradation rate of SMX was 90.4%, and the highest reaction rate constant was 0.0384 min⁻¹¹³. Gao et al. found that *Phanerochaete chrysosporium* has a strong tolerance to sulfamethoxazole in the concentration range of 10–30 mg/L¹⁴. At 10 mg/L, the degradation rate reached 53% after 24 h and 74% after 10 days. Jia et al. reported that the sulfate-reducing bacteria (SRB) sludge system shows considerable ability...
to degrade SMX. When the initial concentration is 25, 50, 100, 150, and 200 mg/L, the removal rates of SMX by SRB sludge through adsorption and biodegradation are 3.9, 5.6, 13.2, 15.9, and 21.3 mg/L/d. Other bacteria, including *Achromobacter denitrificans* PR1, *P. chrysosporium*, *Pseudomonas stutzeri*, and *Acinetobacter sp.*, could serve as resources for the bioremediation of SMX from the polluted environment. Nevertheless, further studies are needed. Previous studies mainly isolated SMX-degrading bacteria through traditional methods, and the degradation efficiency was relatively low. The degradation mechanism of SMX and the metabolic pathway involved are also unclear.

The aims of this study were: (1) to isolate a bacterial strain that can highly degrade SMX by a novel method; (2) to optimize the environmental parameters to improve degradation efficiency; and (3) to deduce the possible degradation pathway of the degrading strain and examine the mechanism underlying SMX degradation.

### Results

**Community changes and diversity of SMX-degrading enrichment cultures.** A total of 5978, 6012, 6048, 6005, and 6003 16S rRNA gene sequences of the distinct V3–V4 regions were obtained from sludge sample (SMXY) and four generations of enrichment cultures (SMX1–4) by high-throughput sequencing, respectively. After statistical analysis and annotation, the results (Fig. 1) showed that 5978 sequences in sludge sample SMXY were clustered into 166 OTUs. The highest abundance of OTUs belonged to bsv13, *Lentimicrobium*, *Flavolibacter*, *Ramlibacter*, and *Rhodoferax*. After enrichment, the bacterial diversity of SMX1 rapidly decreased, 6012 sequences were clustered into only 23 OTUs. The main genera were *Pseudomonas*, *Sphingobacterium*, *Escherichia-Shigella*, *Alcaligenes*, and *Sediminibacter*. *Pseuomonas* became the highest abundance genus, and the proportion of *Sphingobacterium* reached 85.84%. The result indicates that *Sphingobacterium* can tolerate higher concentrations of SMX than the other bacteria. As a result, the diversity in the enrichment gradually declined with the increase in SMX concentrations and passage generations.
Optimization of the degradation conditions for LLE5. The influence of various environmental factors on the biodegradation efficiency was determined. *S. mizutaii* LLE5 can degrade SMX from 15 to 40 °C (Fig. 3a), with an optimal temperature of 30 °C. Although the temperature decreased to 15 °C, the degradation efficiency was more than 42.32%. When the temperature rose to 40 °C, the degradation efficiency was more than 58.65%, and these results showed that *S. mizutaii* LLE5 has an obvious temperature adaptability. pH is another key factor affecting the degradation efficiency of the strain. The results of different pH on the degradation of the strain (Fig. 3b) show that LLE5 can degrade SMX in the pH range 4.0–9.0. At the optimal pH of 7.0, the degradation of SMX was 91.77%. In addition, the degradation efficiency of LLE5 was more than 50% in the pH range 5.0–8.0, which indicated that the degradation efficiency of strain LLE5 was higher under neutral conditions. When the pH was reduced to 4.0, the degradation efficiency was still 48.64%, indicating that the strain was tolerant to acid conditions. When the pH was 9.0, the degradation efficiency was 38.70%, which indicated that LLE5 could be applied under neutral and acid conditions. The different initial inoculum amount on the degradation efficiency was analyzed (Fig. 3c). When the inoculation amount was 5 × 10⁷ cfu/mL, the degradation efficiency of *S. mizutaii* LLE5 was the highest, which was 92.89%. However, when the initial inoculation amount was 2.0 × 10⁷–8.0 × 10⁷ cfu/mL, the degradation efficiency was more than 90%, indicating that the degradation efficiency of *S. mizutaii* LLE5 did not change significantly with the change in inoculum amount. The initial concentration of SMX also influenced the degradation efficiency of *S. mizutaii* LLE5 (Fig. 3d). The degra-
The degradation efficiency of *S. mizutaii* LLE5 with 10 mg/L SMX reached 95.14% and decreased to 83.42% when the SMX concentration reached 300 mg/L. These results indicate that high concentrations of SMX inhibit the growth of *S. mizutaii* LLE5.

The temperature, pH levels and inoculation size were further designed through the response surface method in accordance with the results of single-factor experiments, and 17 degradation tests were carried out (Table 1). By the statistical analysis of data, the following second-degree polynomial equation was obtained to explain SMX biodegradation by *S. mizutaii* LLE5:

\[
Y_1 = 92.74 - 3.09A - 2.06B + 0.32C + 3.10AB - 1.07AC - 1.08BC - 9.40A^2 - 8.40B^2 - 0.12C^2
\]  

where \(Y_1\) represents the SMX degradation efficiency, and A, B, and C are the coded values for temperature, pH level, and inoculum amount respectively. Analysis of variance (ANOVA) for the fitted quadratic polynomial model is shown in Table 2. The model was significant \((P<0.05)\) with \(R^2 = 0.9831\) and Adj \(R^2 = 0.9613\). The results of regression analysis indicated that A, B, AB, A^2 and B^2 are significant model terms, whereas the C, AC, BC, C^2 are nonsignificant model terms. The same was confirmed from the Pareto chart (Fig. 4) in which higher effects were presented in the upper portion and then progress down to the lower effects. It directly shows that the most important factors influencing biodegradation efficiency were A, B, AB, A^2 and B^2. The three-dimensional response surface was plotted to directly display the effects of the temperature and pH level on SMX biodegradation. At the theoretical maximum point of response surface (Fig. 5), the optimum conditions for SMX degradation by *S. mizutaii* LLE5 were 30.8°C, pH 7.2, and inoculum amount of \(3.5 \times 10^7\) cfu/mL.

**Degradation of SMX by strain LLE5.** The degradation characteristics of SMX by *S. mizutaii* LLE5 under the optimal conditions were studied. The results indicated that the most efficient degradation was obtained during the first 3 days. On the third day, the degradation efficiency was 84.02%, and the cell concentration was \(68.95\% \times 10^7\) cfu/mL (Fig. 6). The degradation efficiency was positively correlated with the cell growth density. At 5–7 days, the degradation efficiency of SMX gradually decreased and was accompanied by no further increase in *S. mizutaii* LLE5 cell density. Finally, the degradation efficiency of SMX with initial concentration of 50 mg/L was 93.87% after 7 days. This is the first report that *Sphingobacterium mizutaii* has a good degradation effect on SMX. *S. mizutaii* LLE5 can also degrade other sulfonamides, and the degradation efficiencies of strain LLE5 for sulfadiazine, sulfaguanidine, sulfamisoxazole, and sulfadimidine were 59.85%, 51.68%, 46.95%, and 37.42%, respectively.
respectively (Fig. 7). To elucidate the degradation ability of *S. mizutaii* LLE-5 against various sulfonamides, the degradation constant \(k\) and half-life \(t_{1/2}\) were determined by using the first-order kinetic model. Table 3 presents the kinetics parameters calculated from the model. The coefficient of determination \(R^2\) varied from 0.9572 to 0.9924 indicating that the degradation data reliably fitted with the first-order kinetic model. Degradation rate constants \(k\) varied from 0.0620 to 0.4247 d\(^{-1}\) that characterized the degradation process of various sulfonamides by strain LLE-5. Theoretical half-life \(t_{1/2}\) of SMX, sulfaguanidine, sulfamisoxazole, and sulfadimidine was noted as 1.63, 5.22, 6.80, 7.96, and 11.18 days, respectively. These results show that *S. mizutaii* LLE5 has a broad specificity for the degradation of sulfonamides and has considerable potential for processing sulfonamide pollution in the environment.

**Metabolic pathways of SMX degradation by *S. mizutaii* LLE5.** The metabolites of SMX degraded by *S. mizutaii* LLE5 in MSM liquid medium were detected by HPLC/MS. According to the chemical structure of SMX and the mass spectrum, four candidate products were identified. These products were sulfanilamide (171 m/z), 4-aminothiophenol (124 m/z), 5-amino-3-methylisoxazole (99 m/z), and aniline (92 m/z). None of these products were detected when the culture medium only contained SMX and without *S. mizutaii* LLE5, indicating that they are SMX biodegradation metabolites. A possible metabolic pathway for SMX biodegradation by LLE5 was proposed (Fig. 8). SMX is first transformed into sulfanilamide and 5-amino-3-methylisoxazole through hydrogenation. Then, sulfanilamide is degraded via desulfurization into aniline and via deamination into 4-aminothiophenol. Although the ring-opening products of hydroquinone were not detected, it is still the first report of the pathway of SMX degradation by a *Sphingobacterium* strain. The candidate products were iden-

### Table 1. Box-Behnken experimental design with three independent variables. \(X_1:\) temperature (°C), \(X_2:\) pH level, \(X_3:\) inoculation amount (\(\times 10^7\) cfu/mL).

| Run | \(X_1\) | \(X_2\) | \(X_3\) | Degradation efficiency (%) |
|-----|--------|--------|--------|---------------------------|
| 1   | 25     | 8      | 3      | 72.3                      |
| 2   | 25     | 5      | 3      | 84.8                      |
| 3   | 30     | 6.5    | 3      | 93.11                     |
| 4   | 25     | 6.5    | 1      | 84.5                      |
| 5   | 25     | 6.5    | 5      | 87.1                      |
| 6   | 30     | 8      | 5      | 82.6                      |
| 7   | 30     | 6.5    | 3      | 92.84                     |
| 8   | 35     | 5      | 3      | 71.4                      |
| 9   | 35     | 6.5    | 1      | 81.5                      |
| 10  | 30     | 5      | 5      | 86.7                      |
| 11  | 30     | 8      | 1      | 83.9                      |
| 12  | 30     | 6.5    | 3      | 93.87                     |
| 13  | 35     | 8      | 3      | 71.3                      |
| 14  | 35     | 6.5    | 5      | 79.8                      |
| 15  | 30     | 6.5    | 3      | 92.52                     |
| 16  | 30     | 5      | 1      | 83.7                      |
| 17  | 30     | 6.5    | 3      | 91.37                     |

### Table 2. Analysis of variance (ANOVA) for the fitted quadratic polynomial model. \(P\) Value < 0.05 indicates the model terms are significant.

| Source | Sum of squares | DF | Mean square | F value | \(P\) value |
|--------|----------------|----|-------------|---------|-------------|
| \(X_1\) | 76.26          | 1  | 76.26       | 35.61   | 0.0006      |
| \(X_2\) | 34.03          | 1  | 34.03       | 15.89   | 0.0053      |
| \(X_3\) | 0.85           | 1  | 0.85        | 0.39    | 0.5499      |
| \(X_1X_2\) | 38.44          | 1  | 38.44       | 17.95   | 0.0039      |
| \(X_1X_3\) | 4.62           | 1  | 4.62        | 2.16    | 0.1852      |
| \(X_2X_3\) | 4.62           | 1  | 4.62        | 2.16    | 0.1852      |
| \(X_1X_1\) | 371.73         | 1  | 371.73      | 173.57  | 0.0001      |
| \(X_2X_2\) | 296.81         | 1  | 296.81      | 138.59  | 0.0011      |
| \(X_3X_3\) | 0.062          | 1  | 0.062       | 0.029   | 0.8701      |
| Model | 869.49         | 9  | 96.61       | 45.11   | 0.0002      |
| Error | 3.35           | 4  | 0.84        |         |             |
| Total | 884.48         | 16 |             |         |             |
tified according to their m/z values, SMX chemical properties, and existing reports. Standard samples of four intermediate products were also purchased to conduct parallel experiments.

**Discussion**

*Sphingobacterium* strains widely exist in natural environment. *Sphingobacterium* is a kind of gram-negative bacteria and does not produce spores. Since the genus *Sphingobacterium* was proposed originally by Yabuuchi et al. in 1983, new species have been discovered from a variety of environments, such as soil, plants, animals, and even clinical samples of ventricular fluid and urine. Among them, *Sphingobacterium thalpohilum* and *Sphingobacterium multivarum* can degrade petroleum hydrocarbons. For example, *S. multivorum* SWH-2 has a strong ability to degrade petroleum. After ensuring optimal conditions, the normal growth and enzyme secretion and activity of *S. multivorum* SWH-2 will also change, which can further improve the oil degradation of the strain. In addition, most *Sphingobacterium* found thus far are resistant to a variety of antibiotics from microorganisms. However, there is no report on the direct degradation of antibiotics by *Sphingobacterium*. *Sphingobacterium*
Figure 6. Growth of *S. mizutaii* LLE5 along time and degradation efficiency of SMX. The symbols represent averages of triplicate experiments and the error bars indicate their corresponding standard deviations.

Figure 7. Degradation kinetics of various sulfonamides by *S. mizutaii* LLE5. The symbols represent averages of triplicate experiments and the error bars indicate their corresponding standard deviations.
mizutaii isolated in this study is the first Sphingobacterium reported to degrade SMX. The discovery of this bacterium not only expanded the functional range of Sphingobacterium but also provided valuable microbial resources for the remediation of SMX contaminated aquaculture environment.

In recent years, a number of strains with good SMX degradation ability, such as Planococcus kocurii O516, Achromobacter sp. JL9, Phanerochaete chrysosporium, and Acinetobacter sp., have been isolated from soil, activated sludge, and aquaculture water. Compared with the reported strain, the strain LLE-5 had a better environmental range and tolerance. The study on the influence of environmental factors on the degradation effect indicates that strain LLE-5 can degrade SMX at the temperature range 15–40 °C and at pH 4–9. This finding provides a basis for the practical application of the strain in the future. The physiological and biochemical results

| Sulfonamides          | Regression equation | k (d⁻¹) | t₁/₂ (d) | R²         |
|-----------------------|---------------------|---------|----------|------------|
| Sulfamethoxazole      | Cₜ = 51.3514 × e⁻⁰.⁹⁵₅₄ | 0.4247  | 1.63     | 0.9887     |
| Sulfadiazine          | Cₜ = 50.6351 × e⁻⁰.⁸₂₂₇ | 0.1327  | 5.22     | 0.9924     |
| Sulfaguanidine        | Cₜ = 50.2014 × e⁻⁰.⁹₈₉₈ | 0.1019  | 6.80     | 0.9805     |
| Sulfamisoxazole       | Cₜ = 49.8941 × e⁻⁰.⁸₀₇₃ | 0.0871  | 7.96     | 0.9572     |
| Sulfadimidine         | Cₜ = 50.1347 × e⁻⁰.⁶₂₅₉ | 0.0620  | 11.18    | 0.9716     |

Table 3. Kinetic parameters of various sulfonamides degradation by S. mizutaii LLE-5. k represents degradation constant (d⁻¹); t₁/₂ represents half-time (d); R² represents correlation coefficient; Ct is the concentration (mg/L) of sulfonamides at time t.

Figure 8. Proposed pathway for the degradation of SMX by S. mizutaii LLE5.
showed that LLE-5 could use a variety of carbon sources. The bacteria have strong adaptability to the environment, rich types of nutrition metabolism, and potential to degrade other complex compounds.

The existing reports show that most strains of *Sphingobacterium* cannot produce antibiotics but are resistant to antibiotics, which is an important factor for the *Sphingobacterium* to adapt to extreme environments. Antibiotics are compounds that inhibit the growth of microorganisms. Thus, degrading bacteria need to have antibiotic resistance. Some studies have shown that *Klebsiella pneumoniae* can use chloramphenicol as the sole carbon source for growth. However, the drug sensitivity test showed that these strains were sensitive to chloramphenicol, which indicated that the drug resistance and drug degradation processes were two independent pathways. Drug-resistant bacteria can only resist antibiotics and avoid growth inhibition. By contrast, antibiotic-degrading bacteria resist antibiotics and produce degrading enzymes such as monoxygenase, esterase, or hydrolase to degrade the drug. We sequenced the whole genome of strain LLE5 and found some genes that might be involved in SMX degradation (data not shown). Their functions will be identified in our future work. According to the degradation products of SMX by *S. mizutai* LLE-5, the strain mainly transformed SMX into sulfanilamide and 5-amino-3-methylisoxazole through hydrogenation. Then, sulfanilamide is degraded via desulfurization into aniline and via deamination into 4-aminothiophenol. The results showed that the strain had a clear degradation function of sulfamethoxazole and had a good tolerance. However, SMX could not be completely mineralized in terms of degradation products. This work is the first to report the pathway of SMX degradation by a *Sphingobacterium* strain and provided a certain reference for the study of microbial degradation of SMX.

**Methods**

**Chemicals and media.** SMX (analytical standard, >99.5%), HPLC-grade methanol, and acetonitrile were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. All other reagents used in the present study were of analytical grade. Mineral salt medium (MSM) and Luria–Bertani (LB) medium were used as described by Song et al. Agar plates were prepared by adding 1.5% (w/v) agar into the liquid media.

**Enrichment culture and high-throughput sequencing.** Activated sludge samples were collected from the wastewater treatment pond of a pharmaceutical factory in Liaoang City, Liaoang Province, China. 10.0 g of sample was suspended in 100 mL of MSM supplemented with 100 mg/L SMX. Then, the sample was incubated on a rotary incubator shaker at 30 °C and 100 rpm in the dark. After 7 days, 10 mL of the sample was transferred into fresh MSM supplemented with 200 mg/L of SMX and incubated for another 7 days. After repeating this process four times, the SMX concentration in the fourth generation increased to 500 mg/L. The activated sludge sample was designated as SMX-Y, and the four generations of SMX enrichment cultures were designated as SMX1, SMX2, SMX3, and SMX4. The total DNA of SMX-Y and SMX1-4 were extracted using a FastDNA SPIN kit for soil (MP, Biomedicals, USA). After purification, the 16S rRNA distinct regions V3–V4 were amplified with the following primers: 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGYTWTCTAAAT-3′). The polymerase chain reaction (PCR) system was designed as described by Li et al. The PCR product was sent to Biomark Gene Technology Company for library construction and high-throughput sequencing. The obtained sequence data were analyzed using Arch software (http://www.drive5.com/usearch/). The identity of sequences ≥97% was assigned to an operational taxonomic unit (OTU), and each OTU was considered to represent a species. The relative abundance of OTUs in the samples was statistically analyzed using R software (https://www.R-project.org).

**Isolation and identification of SMX-degrading bacteria.** According to the results of high-throughput sequencing, the fourth-generation enrichment sample SMX4 containing the most *Sphingobacterium* cells was used for isolation of SMX-degrading bacteria. Then, 100 μL of SMX4 was spread on the MSM solid plate containing 100 mg/L SMX and incubated at 30 °C. The rapidly growing colonies on the plate with different morphologies were selected and restreaked three times to obtain pure cultures. The degradation efficiency of the isolates was determined by a high-performance liquid chromatography (HPLC) system (1260, Agilent, Santa Clara, CA) equipped with an eclipse Plus C18 column. 10 mL of the culture and equal volume of ethyl acetate were added into a 50 mL centrifuge tube and mixed using a vortex mixer for 1 min. Then, the centrifuge tube was placed in a shaker, mixed at 220 r/min for 30 min, and centrifuged at 5000 g for 8 min. The upper phase was filtered with a 0.22 μm membrane for HPLC analysis. The elution comprised a mixture of methanol, formic acid (85/15/0.1, v/v/v), and distilled water, running at the flow rate of 1.0 mL/min. The injection volume was 10 μL, and the column temperature was 30°C. The isolate with the highest degradation efficiency was selected for further analysis. Morphology was investigated using a light microscope (BX-51; Olympus, Japan). The carbohydrate assimilation of isolate was conducted with Biolog GEN3 plates following the analytical methods described by Hobbie et al. The genomic DNA of the isolate was extracted and was used as a template for 16S rDNA amplification as described by Ruan et al. The universal primers were 27F (5′-AGAGTTTGTATCCTGGCTCAG-3′) and 1492R (5′-ACGGTACCTGTTCAGACTT-3′). The purified PCR product was sequenced by Bomad Technology (Beijing, China). The obtained sequence was deposited in GenBank. The strain was further identified by performing multiple sequence alignment using Clustal X software, and phylogenetic relationships were analyzed via the neighbor-joining (NJ) method with MEGA 6 software.

**Inoculum preparation.** Before the degradation experiment, strain activation was performed. *S. mizutai* LLE-5 was inoculated into 100 mL of LB medium and incubated at 30 °C on a rotary shaker at 150 rpm. After 12 h, the bacterial cells were harvested by centrifugation at 4000 g for 10 min. The precipitate was washed two times by phosphate buffered saline (PBS) solution and suspended for subsequent studies.

9. **https://doi.org/10.1038/s41598-021-02404-x**
Optimization of the SMX-degrading conditions. The effect of single environmental factors on the biodegradation efficiency was evaluated by analyzing the following parameters and their ranges: temperature (15 °C, 20 °C, 25 °C, 30 °C, 35 °C, and 40 °C); initial medium pH (4, 5, 6, 7, 8, and 9); inoculum amount (1.0 × 10^7, 2.0 × 10^7, 3.0 × 10^7, 5.0 × 10^7, 8.0 × 10^7, and 10.0 × 10^7 cfu/mL); and initial concentration (10, 50, 100, 200, 300 and 500 mg/L). The sterile distilled water was supplemented every 12 h according to the loss of weight. Selected single environmental factors and their interactions were further optimized via response surface methodology (RSM) analysis based on Box–Behnken design. The second order polynomial equation is expressed as follows:

\[ Y_i = b_0 + \sum b_{ij}X_i + \sum b_{ij}X_j + \sum b_{ijk}X_i^2 \]  

where \( Y_i \) refers to the predicted response, \( X_i \) and \( X_j \) are variables, \( b_i \) is a constant, \( b_j \) denotes the linear coefficient, \( b_{ij} \) represents the quadratic coefficient, and \( b_{ij} \) corresponds to the interaction coefficient.

Biodegradation tests. The SMX degradation test was performed under optimal conditions. S. mizutaii LLE-5 cells were inoculated into 100 mL of MSM supplemented with 50 mg/L of SMX. The residual of SMX and cell numbers of S. mizutaii LLE-5 were detected every day. The control was inoculated with killed cells of S. mizutaii LLE-5. Each test was conducted in triplicate. The ability of S. mizutaii LLE-5 to degrade other structurally similar sulfonamides, including sulfadiazine, sulfaguanidine, sulfamisoxazole, and sulfadimidine, were evaluated. Analytical methods were the same as above described. The first-order kinetic model (Eq. 3) was created to elucidate sulfonamides degradation efficiency of S. mizutaii LLE-5:

\[ C_t = C_0 \times e^{-kt} \]  

where \( C_0 \) is the initial concentration of sulfonamides at time zero, \( C_t \) is the concentration of sulfonamides at time \( t \), \( k \) is the degradation rate constant (d⁻¹).

The theoretical half-life (t₁/₂) values of different sulfonamides were calculated by Eq. (4):

\[ t_{1/2} = \frac{\ln(2)}{k} \]  

where \( \ln 2 \) is the natural logarithm of 2 and \( k \) is degradation rate constant (d⁻¹).

Identification of SMX biodegradation intermediates. Intermediates generated during SMX degradation by S. mizutaii LLE-5 in MSM were analyzed by HPLC–MS (AB Sciex QTRAP 5500, USA). The extraction method was same as described above. The flow rate was 0.2 mL/min, the mobile phase A was 0.1% formic acid (V/V), and B was methanol. The targeted screening gradient elution program was: 0–2 min, 95% A; 2–25 min, 95%–5% A; 25–35 min, 5% A; and 35–40 min, 95% A. The sample injection volume was 20 μL, and the column temperature was 30 °C. The mass spectrometry conditions were: DuosprayTM ion source, electrospray ionization (ESI), positive ion mode scanning, the ion source temperature was 550 °C, the spray voltage was 5500 V, and curtain air (CUR) was 35 psi. The accumulation time was 0.25 s, and the collision voltage was (35 ± 15) eV.

Received: 3 August 2021; Accepted: 6 October 2021
Published online: 30 November 2021

References
1. Han, Q. F. et al. Distribution, combined pollution and risk assessment of antibiotics in typical marine aquaculture farms surrounding the Yellow Sea, North China. Environ. Int. 138, 105551 (2020).
2. Zhang, L. et al. Biodegradation mechanisms of sulfonamides by Phanerochaete chrysosporium: Luffa fiber system revealed at the transcriptome level. C hemosphere 266, 129194 (2021).
3. Shah, S. et al. Recent advances in medicinal chemistry of sulfonamides: Rational design as anti-tumoral, anti-bacterial and anti-inflammatory agents. Mini. Rev. Med. Chem. 13, 70–86 (2013).
4. Cribb, A. & Spielberg, S. Sulfamethoxazole is metabolized to the hydroxylamine in humans. Clin. Pharmacol. Ther. 51, 522–526 (1992).
5. Mulla, S. I. et al. Biodegradation of sulfamethoxazole in bacteria from three different origins. J. Environ. Manage. 206, 93–102 (2018).
6. Su, T., Deng, H., Benskin, J. P. & Radke, M. Biodegradation of sulfamethoxazole photo-transformation products in a water/sediment test. Chemosphere 148, 518–525 (2016).
7. Xiong, J. Q. et al. Combined effects of sulfamethazine and sulfamethoxazole on a freshwater microalgae, Scenedesmus obliquus: Toxicity, biodegradation, and metabolic fate. J. Hazard Mater. 370, 138–146 (2019).
8. Li, T. et al. Enhanced degradation of sulfamethoxazole by a novel Fenton-like system with significantly reduced consumption of H₂O₂ activated by g-C₃N₄/MgO composite. Water Res. 190, 116777 (2021).
9. Goncalves, M. G. et al. Relationship of the physicochemical properties of novel ZnO/biochar composites to their efficiencies in the degradation of sulfamethoxazole and methyl orange. Sci. Total Environ. 748, 141381 (2021).
10. Hong, M., Wang, Y. & Lu, G. UV-Fenton degradation of diclofenac, sulpiride, sulfamethoxazole and sulfisomidine: Degradation mechanisms, transformation products, toxicity evolution and effect of real water matrix. Chemosphere 258, 127351 (2020).
11. Carneiro, R. B. et al. Influence of organic loading rate on ciprofloxacin and sulfamethoxazole biodegradation in anaerobic fixed bed biofilm reactors. J. Environ. Manage. 273, 111170 (2020).
12. Rodrigues, D. et al. Biodegradation of sulfamethoxazole by microalgae-bacteria consortium in wastewater treatment plant effluents. Sci. Total Environ. 749, 141441 (2020).
13. Liang, D. H. & Hu, Y. Simultaneous sulfamethoxazole biodegradation and nitrogen conversion by Achromobacter sp. JL9 using different carbon and nitrogen sources. Bioresour. Technol. 293, 122061 (2019).
14. Gao, N. et al. Simultaneous removal of ciprofloxacin, norfloxacin, sulfamethoxazole by co-producing oxidative enzymes system of \textit{Phanerochaete chrysosporium} and \textit{Pseudomonas sargentii}. \textit{Chemosphere} \textbf{195}, 146–153 (2018).
15. Jia, Y. et al. Sulfamethoxazole degradation in anaerobic sulfate-reducing bacteria sludge system. \textit{Water Res.} \textbf{119}, 12–20 (2017).
16. Reis, P. J. et al. Biodegradation of sulfamethoxazole and other sulfonamides by \textit{Achromobacter dennisificans} PRL. \textit{J. Hazard Mater.} \textbf{280}, 741–749 (2014).
17. Jiang, B. et al. Biodegradation and metabolic pathway of sulfamethoxazole by \textit{Pseudomonas psychrophila} HA-4, a newly isolated cold-adapted sulfamethoxazole-degrading bacterium. \textit{Appl. Microbiol. Biotechnol.} \textbf{98}(10), 4671–4681 (2014).
18. Yang, F. et al. Effects of biochar on biodegradation of sulfamethoxazole and chloramphenicol by \textit{Pseudomonas stutzeri} and \textit{Shewanella putrefaciens}: Microbial growth, fatty acids, and the expression quantity of genes. \textit{J. Hazard Mater.} \textbf{406}, 124311 (2021).
19. Wang, S. & Wang, J. Biodegradation and metabolic pathway of sulfamethoxazole by a novel strain \textit{Acinetobacter sp.} \textit{Appl. Microbiol. Biotechnol.} \textbf{102}(1), 425–432 (2018).
20. Naka, T. et al. Structural analysis of sphingophospholipids derived from \textit{Sphingobacterium spiritorum}, the type species of genus \textit{Sphingobacterium}. \textit{Biochim. Biophys. Acta.} \textbf{1635}(2–3), 83–92 (2003).
21. Wauters, G. et al. Isolates belonging to CDC group II-i belong predominantly to \textit{Sphingobacterium mistizutai} Yabuuchi et al. 1983: Emended descriptions of \textit{S. mistizutai} and of the genus \textit{Sphingobacterium}. \textit{Int. J. Syst. Evol. Microbiol.} \textbf{62}(Pt 11), 2598–2601 (2012).
22. Alene, G. et al. \textit{Sphingobacterium multivorum} isolation, characterization, and activity in a composting system. \textit{Appl. Eng. Biosaf.} \textbf{33}(9), e387502016 (2016).
23. Barahona, F. & Slim, J. \textit{Sphingobacterium multivorum}: Case report and literature review. \textit{New Microbes New Infect.} \textbf{7}, 33–36 (2015).
24. Shirinivasan, V. B., Vaidyanathan, V., Mondal, A. & Rajamohan, G. Role of the two component signal transduction system CpxAR in conferring cefepime and chloramphenicol resistance in \textit{Klebsiella pneumoniae} NTUH-K2044. \textit{PLoS ONE} \textbf{7}(4), e33777 (2012).
25. Song, J. et al. Pathway and kinetics of malachite green biodegradation by \textit{Pseudomonas veronii}. \textit{Sci. Rep.} \textbf{10}(1), 4502 (2020).
26. Li, M. et al. Insight into the characteristics and new mechanism of nicosulfuron biodegradation by a \textit{Pseudomonas sp.} LAM1902. \textit{J. Agric. Food Chem.} \textbf{68}(3), 826–837 (2020).
27. Zhou, S. et al. Nicosulfuron biodegradation by a novel cold-adapted strain \textit{Oceanisphaera psychrotolerans} LAM-WHM-ZC. \textit{J. Agric. Food Chem.} \textbf{65}(47), 10243–10249 (2017).
28. Larcher, S. & Vargau, V. Biodegradation of sulfamethoxazole by individual and mixed bacteria. \textit{Appl. Microbiol. Biotechnol.} \textbf{91}(1), 211–218 (2011).
29. Hobbie, E. A. et al. Carbohydrate use and assimilation by litter and soil fungi assessed by carbon isotopes and BIOLOG assays. \textit{Soil Biol. Biochem.} \textbf{35}, 303–311 (2003).
30. Ruan, Z. et al. Isolation and characterization of a novel cinosulfuron degrading \textit{Kanthia sp.} from a methanogenic microbial consortium. \textit{Bioresour. Technol.} \textbf{147}, 477–483 (2013).
31. Ruane, N. M. et al. Isolation of \textit{Streptococcus agalactiae} and an aquatic birnavirus from doctor fish \textit{Garra rufa} L. \textit{Ir. Vet. J.} \textbf{66}(1), 16 (2013).
32. He, Z. et al. Biodegradation of feather waste keratin by the keratin-degrading strain \textit{Bacillus subtilis} 8. \textit{J. Microbiol. Biotechnol.} \textbf{28}(2), 314–322 (2018).
33. Wang, S., Zhang, C. & Yan, Y. Biodegradation of methyl parathion and p-nitrophenol by a newly isolated \textit{Agrobacterium sp.} strain Yw12. \textit{Biodegradation} \textbf{23}(1), 107–116 (2012).
34. Wang, W. et al. Optimization of reactions between reducing sugars and 1-phenyl-3-methyl-5-pyrazolone (PMP) by response surface methodology. \textit{Food Chem.} \textbf{254}, 158–164 (2018).
35. Bhatt, P., Huang, Y., Zhan, H. & Chen, S. Insight into microbial applications for the biodegradation of pyrethroid insecticides. \textit{Front Microbiol.} \textbf{10}, 1778 (2019).
36. Cycon, M., Zmijowska, A. & Piotrowska-Seget, Z. Enhancement of deltamethrin degradation by soil bioaugmentation with two different strains of \textit{Serratiamarcescens}. \textit{Int. Environ. Sci. Technol.} \textbf{11}, 1305–1316 (2014).

Acknowledgements
This work was supported by Open Research Fund of Key Laboratory of Healthy Freshwater Aquaculture, Ministry of Agriculture and Rural Affairs (ZJK202101), Central Public-interest Scientific Institution Basel Research Fund, (CAFS, No. 2021A003) and National Key Research and Development Program of China (No. 2017YFC1600704).

Author contributions
Y.M. and Z.R. conceived and designed research. J.S., G.H., L.L., H.Z., and D.Z. conducted experiments. J.X., X.L. and Z. Y. analyzed and discussed the data. J.S. wrote the manuscript. All authors read and approved the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-02404-x.

Correspondence and requests for materials should be addressed to Z.R. or Y.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
