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ORIGINAL PAPER

Biodegradation of sulfamethazine by an isolated thermophile–*Geobacillus* sp. S-07

Lan-jia Pan[1,2] · Xiao-da Tang[1,2] · Chun-xing Li[1] · Guang-wei Yu[1] · Yin Wang[1]

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Abstract Sulfamethazine (SM2) is an antimicrobial drug that is frequently detected in manure compost, is difficult to degrade at high temperatures and is potentially threatening to the environment. In this study, a thermophilic bacterium was isolated from the activated sludge of an antibiotics pharmaceutical factory; this bacterium has the ability to degrade SM2 at 70 °C, which is higher than the traditional manure composting temperature. The strain S-07 is closely related to *Geobacillus thermoleovorans* based on its 16S rRNA gene sequence. The optimal conditions for the degradation of SM2 are 70 °C, pH 6.0, 50 rpm rotation speed and 50 mL of culture volume. More than 95% of the SM2 contained in media was removed via co-metabolism within 24 h, which was a much higher percentage than that of the type strain of *G. thermoleovorans*. The supernatant from the S-07 culture grown in SM2-containing media showed slightly attenuated antibacterial activity. In addition, strain S-07 was able to degrade other sulfonamides, including sulfadiazine, sulfamethoxazole and sulfamerazine. These results imply that strain S-07 might be a new auxiliary bacterial resource for the biodegradation of sulfonamide residue in manure composting.

Keywords Antibiotics · Biodegradation · Thermophilic microorganism · Acclimation · Co-metabolism

Introduction

Sulfonamides, as a group of synthetic antibiotics, have been used as antimicrobial drugs for more than 70 years (Dmitrienko et al. 2014). These drugs are commonly used in livestock and poultry breeding to prevent diseases and to treat illnesses. However, it is estimated that approximately 30–90% is excreted in manure (Heuer et al. 2011), resulting in a high concentration of sulfonamide residue in manure. The antibiotic concentrations in manure range from trace levels to >200 mg/kg, and the typical concentrations are in the range of 1–10 mg/kg (Ramaswamy et al. 2010). Sulfathiazole and sulfamethazine (SM2) residues were detected in manure at concentrations of 12.4 and 8.7 mg/kg, respectively (García-Galán et al. 2008). Even the particularly high concentration of 500 mg/L of sulfadiazine in slurry was reported (Grote et al. 2004).

Composting, as an effective bioremediation strategy for manure contamination, has been proposed as a feasible approach to promote the removal of antibiotics from animal manure (Wu et al. 2011; Selvam et al. 2013). However, traditional composting (the highest composting temperature is approximately 65 °C) cannot remove all antibiotic residue. Sulfonamides are recalcitrant to natural biodegradation (Zhang et al. 2012), exhibiting high thermal stability. Degradation of SM2 was not observed after 35 days of composting (Dolliver et al. 2008). Van Dijk and Keukens (2000) reported that sulfachloropyrazine concentrations in poultry manure decreased by 58–82% during 8 days of composting. Despite the sulfonamide removal being more than 90% in some composting tests, residual sulfonamides

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✉ Yin Wang
yinwang@iue.ac.cn

[1] CAS Key Laboratory of Urban Pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

still remained in the composting product (Mitchell et al. 2015).

The residue of sulfonamides and their metabolites in manure can easily enter the terrestrial environment through soil amendment, which can lead to a series of ecological problems. They can be taken up by plants in manure-amended soil (Boxall et al. 2006; Dolliver et al. 2007). Moreover, sulfonamides in terrestrial environments show a high potential to resist degradation and are hydrophilic enough to be transferred into aquatic environments, which causes their frequent detection in surface and ground waters (Watts et al. 1982; Hirsch et al. 1999; Batt et al. 2006a, b; Kim et al. 2007). Residual sulfonamides could serve as a selecting pressure for the development of antibiotic-resistant bacteria (Zhang et al. 2012). Sulfonamide-resistant bacteria and genes are prevalent in various environments (Gao et al. 2012, Hsu et al. 2014). These facts manifest the impact of residual sulfonamides on human health and reinforce the need to develop effective methods to degrade sulfonamides.

Temperature and microorganisms are essential in the removal of contaminants during composting. Complete removal of antibiotic residue in manure could be achieved by increasing the composting temperature and/or number of inoculating microorganisms that can specifically degrade antibiotics under certain conditions in manure composting system. In recent years, an increasing number of bacteria with the ability to degrade sulfonamides have been isolated (Zhang et al. 2012; Topp et al. 2013; Jiang et al. 2014; Reis et al. 2014). However, most of these bacteria grow at room temperature, which is not suitable for high-temperature composting. This study aims to isolate special microorganisms that can survive at temperatures higher than traditional values and can efficiently degrade sulfonamides. By using acclimation and enrichment techniques, a thermophilic bacterium with the ability to degrade SM2 at 70 °C was isolated and designated S-07; the optimal conditions for bacterial growth and biodegradation of SM2 were subsequently characterized. The residual antibacterial activity of SM2 metabolites was also investigated to identify a reduction in toxicity.

Materials and methods

Chemicals and media

The reagent-grade sodium salt of SM2 and the SM2 analytical standard were obtained from TCI Development Co. Ltd. (Shanghai, China) and Aladdin Industrial Corporation (Shanghai, China), respectively. Sulfamerazine (SM1), sulfadiazine (SD) and sulfamethoxazole (SMX) were purchased from Sigma–Aldrich (USA), and yeast extract was obtained from Sangon Biotech Co. Ltd. (Shanghai, China). Acetonitrile and methanol (HPLC grade) were bought from Fisher (USA). All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd. (China).

Minimal mineral salt medium (MMSM) (Larcher and Yargeau 2011) containing $Na_2EDTA{\cdot}2H_2O$ (0.018 g/L), $FeSO_4{\cdot}7H_2O$ (0.013 g/L), $CaCl_2{\cdot}2H_2O$ (0.013 g/L), $MgSO_4{\cdot}7H_2O$ (0.25 g/L), Na_2HPO_4 (7.5 g/L), KH_2PO_4 (5 g/L), NH_4NO_3 (5 g/L), and yeast extract (0.6 g/L) was supplemented with 10 mg/L of SM2 and 0.5 g/L of glucose (an external easily degradable carbon source). The Luria–Bertani medium (LB) (10 g/L of tryptone, 5 g/L of yeast extract, and 10 g/L of NaCl) was used for cell growth. To prepare solid medium, 1.5–2.0% (w/v) agar was added. All media were sterilized at 120 °C for 20 min prior to use.

Strain isolation and identification

Activated sludge was obtained from an antibiotics pharmaceutical factory in China. To enrich the thermophilic sulfonamide-degrading bacteria, 5 g of sludge was added to 100 mL of MMSM medium with 5 mg/L of the sodium salt of SM2. The culture was placed on a dark rotary shaker (100 rpm) at 70 °C for 10 days, and then the supernatant from the enrichment culture was centrifuged and transferred into fresh sterile MMSM medium containing 10 mg/L of the SM2 sodium salt. The inoculation steps were repeated until the SM2 concentration in the medium was 50 mg/L. Stepwise acclimation of the enriched culture was conducted to ensure the maintenance of bacterial degradation. Enriched culture was 10-fold serially diluted and spread onto MMSM agar plates containing 50 mg/L of SM2. Bacterial colonies were streaked for purification and tested for SM2-degrading capability.

One strain, designated S-07, with SM2-degrading ability was purified and selected for further investigation. The type strain of *Geobacillus thermoleovorans* (strain number 1.3474) was purchased from the China General Microbiological Culture Collection Center (Table S2).

Bacterial DNA was extracted using the Bacterial Genome DNA Extraction Kit (Shanghai Generay Biotech Co., Ltd.) according to the manufacturer's instructions. The 16S rRNA gene was amplified using universal primers 27f (5′-AGAGTTTGATTCTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′). The amplification reaction mixtures (25 μL) contained 12.5 μL of 2X Taq Master Mix, 1 μL of each primer, 1 μL of DNA template and 9.5 μL of ddH_2O. The amplification was performed using a Mastercycler gradient (Eppendorf, Germany) with the following program: denaturation for 5 min at 94 °C; then 30 cycles consisting of 94 °C for 40 s, 55 °C for 40 s, and 72 °C for 100 s; and a final extension step at 72 °C for

10 min. Amplification products were analyzed by electrophoresis on a 2.0% agarose (Sigma, USA) gel, and the PCR products were sequenced at MajorBio (Shanghai, China). The sequence was compared against the available DNA sequences in GenBank (http://www.ncbi.nlm.nih.gov/) using the BLASTN tool (BLASTN 2.6.0). After alignment with different 16S rRNA gene sequences from GenBank, phylogenesis was analyzed using MEGA version 4.0 software, and a phylogenetic tree was constructed using a neighbor-joining method.

HPLC analysis

Aliquot cultures (1000 µL) were filtered through a 0.45-µm membrane filter to remove bacterial cells and particles and then stored in amber vials at 4 °C for further analysis. The sulfonamide concentration was determined using a high-performance liquid chromatography instrument (HPLC, Hitachi L-2000, Japan) equipped with an auto sampler. Chromatographic separation was achieved on an Extend-C18 column (250×4.6 mm, 5-µm 80 A) from Agilent (USA). The SM2 was detected with 0.04 M ammonium acetate and methanol (65:35, v/v) as the mobile phase. The wavelength and the column temperature were set to 264 nm and 30 °C, respectively. For the detection of SM1, SD and SMX, the testing procedure was identical to that of SM2, only with different mobile phases and wavelengths (0.3% formic acid and methanol (79:21, v/v) at 270 nm for SM1; 0.05 M $NH_4H_2PO_4$ and acetonitrile (80:20, v/v) at 270 nm for SD; 0.02 M $NH_4H_2PO_4$ (pH = 2.8) and acetonitrile (70:30, v/v) at 245 nm for SMX). The column was equilibrated for 10–20 min prior to injection.

Degradation of SM2 by strain S-07 and type strain of *Geobacillus thermoleovorans*

Strain S-07 was grown to the exponential growth phase (approximately 12 h) in 50 mL of LB medium at 70 °C and 100 rpm. Bacterial cells were collected after centrifugation at 6000 rpm for 5 min at room temperature. The cell pellets were washed twice with sterilized 0.1 M phosphate buffer (pH = 7.0) and resuspended in sterilized 0.9% NaCl solution to a concentration of $OD_{600} = 2.0$. The bacterial cell suspensions were inoculated at 1.0% (v/v) into MMSM liquid medium supplemented with 10 mg/L of SM2. To determine the optimal conditions for SM2 degradation, the cultures were incubated at various temperatures (50, 55, 60, 65, 70 and 75 °C) with an initial pH of 6.5, or various initial pH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) at the determined optimal temperature. The experiments with different rotation speeds (0, 50, 100 and 150 rpm) and culture volumes (30, 40 and 50 mL using a 100-mL Erlenmeyer flask) were prepared at the determined optimal temperature and pH for 24 h on a dark rotary shaker. Sterilized water was added instead of strain S-07 in control experiments. Based on preliminary experiments and references, the sorption of SM2 onto biomass or other materials was negligible (Herzog et al. 2013). All treatments were performed in triplicate in the dark to avoid photodegradation. Because of the high temperature, the evaporation of moisture was adjusted by weighing prior to sampling. The SM2 degradation by the type strain of *G. thermoleovorans* was measured as abovementioned.

Antibacterial activity of SM2 and its metabolites

To evaluate the antibacterial activity of SM2 and its metabolites, the biomass of the microorganism (*Escherichia coli* K12) exposed to SM2/metabolites was measured. *E. coli* K12 was cultured in LB media at 37 °C on a rotary shaker for 24 h as the seed liquid. The supernatant from S-07 cultured in the presence of SM2 was collected and subsequently filtered with a 0.22-µm membrane three times to remove the biomass and other particles. The filtered culture and SM2 solution (10 mg/L) were added to the LB medium inoculated with *E. coli* K12 seed liquid. An equal volume of MMSM (treated at 70 °C on a shaker for 24 h) was added as a control. The growth of *E. coli* K12 after a 24-h incubation was determined using a UV-1100 ultraviolet spectrophotometer (Mapada, China) at a wavelength of 600 nm. The microbial growth rate of the control was considered to be 100%. The relative growth rates of the supernatant from the S-07 culture supplemented with SM2 at 70 °C for 24 h (E1) and the medium (MMSM cultured S-07 without SM2 for 24 h at 70 °C) containing SM2 (E2) were expressed as a percentage of the control. The antibacterial activity of the liquid was inversely proportional to the relative growth rate.

Degradation of other sulfonamides

The ability of strain S-07 to degrade SD, SMX and SM1 was examined in MMSM containing 10 mg/L of each compound, with the control lacking inoculation of strain S-07. The samples were collected every 24 h to examine the residual concentration of the three compounds.

Results

Isolation and identification of the SM2-degrading strain

After acclimation and selective enrichment with SM2, a bacterial strain, S-07, was obtained with the ability to grow on solid MMSM medium supplemented with 50 mg/L of SM2. Analysis of the partial 16S rRNA gene sequence

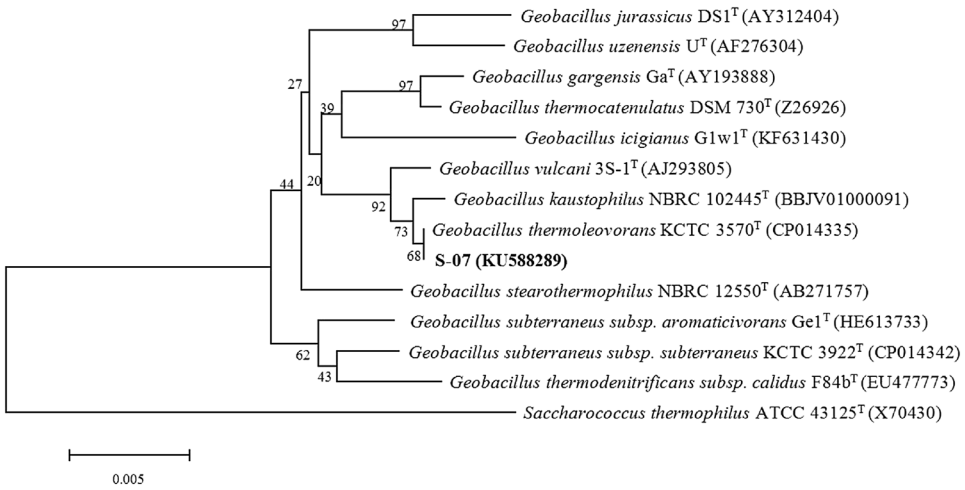

Fig. 1 Phylogenetic tree constructed using the neighbor-joining method based on the 16S rRNA gene sequences of strain S-07 and associated strains

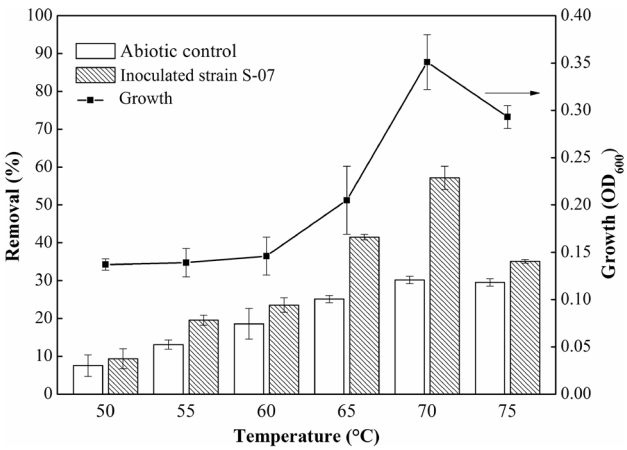

Fig. 2 Effect of temperature on the growth of strain S-07 and the degradation of SM2 (pH = 6.5, 100 rpm). Data are presented as the average value of triplicate measurements with standard deviation

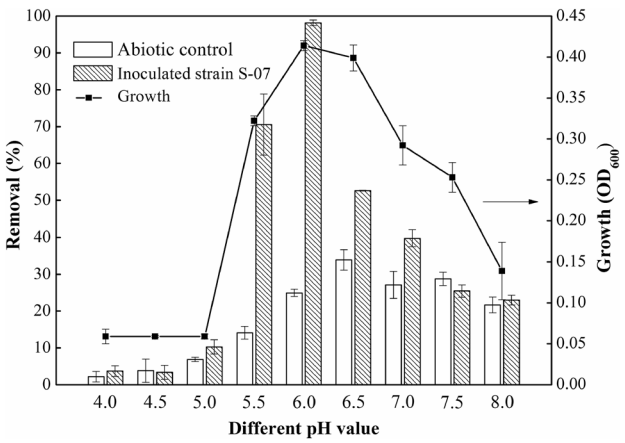

Fig. 3 Effect of pH on the growth of strain S-07 and the degradation of SM2 (70 °C, 100 rpm). Data are presented as the average value of triplicate measurements with standard deviation

of strain S-07 (1303 bp, GenBank accession number: KU588289) indicated an affiliation with the genus *Geobacillus* and showed 100% similarity to that of *G. thermoleovorans* KCTC 3570T (GenBank accession number CP014335) (Fig. 1).

Optimal conditions for biodegradation of SM2 by strain S-07

The growth of strain S-07 and the degradation of SM2 occurred over a wide range of temperatures. Strain S-07 grew well in a temperature range of 50–75 °C (Fig. 2). SM2 degradation increased with temperature from 50 to 70 °C and reached a maximum value of 57.15% at 70 °C. However, it decreased to 35.09% when the temperature increased from 70 to 75 °C. Bacterial growth was consistent with the degradation of SM2.

Bacterial growth and degradation of SM2 increased with the increase of initial pH, peaking at 6.0. The highest bacterial population and degradation were 0.414 (OD_{600}) and 98.18%, respectively. In a pH range from 4.0 to 5.0, low bacterial growth and degradation were observed. When the pH value reached 5.5, both the bacterial growth and degradation of SM2 significantly increased. When the pH value was above 7.5, strain S-07 seemed to have no effect on SM2 degradation. Based on the obtained results, slightly acidic pH values are more favorable for SM2 degradation (Fig. 3).

Strain S-07 showed similar growth and SM2-degrading ability at 0, 50 and 100 rpm, with over 95% SM2 being removed (Fig. 4). However, despite the large bacterial biomass at 150 rpm, SM2 degradation significantly decreased to a level similar to that of the control. In the culture volume test, the SM2 degradation reached a maximum value of 94.66% after 24 h in a 100-mL Erlenmeyer flask

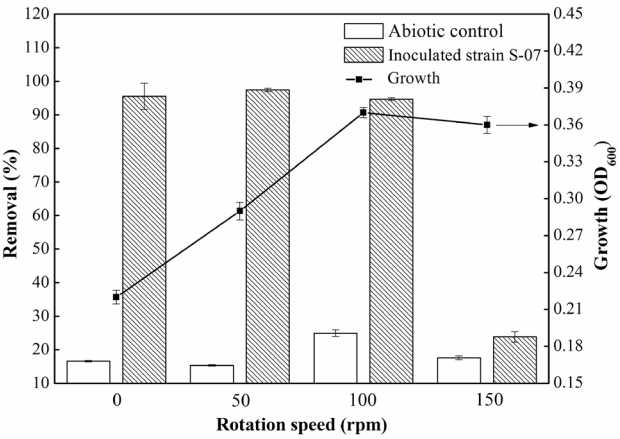

Fig. 4 Effect of rotation speed on the growth of strain S-07 and the degradation of SM2 (70 °C, pH = 6.0). Data are presented as the average value of triplicate measurements with standard deviation

containing 50 mL of MMSM. When the culture volume was decreased to 30 mL, the degradation of SM2 strikingly decreased to <40% (Fig. 5).

Biodegradation of SM2 by type strain of *Geobacillus thermoleovorans*

To test whether SM2 biodegradation is a characteristic of the genus, the SM2 degradation by the type strain of *G. thermoleovorans* was investigated in MMSM medium containing 10 mg/L of SM2. The result showed that only 29.98% (including abiotic effects) of SM2 was removed at 70 °C after 24 h and 36.37% after 48 h. Compared with strain S-07, the degradation capability of the *G. thermoleovorans* type strain for SM2 was much lower.

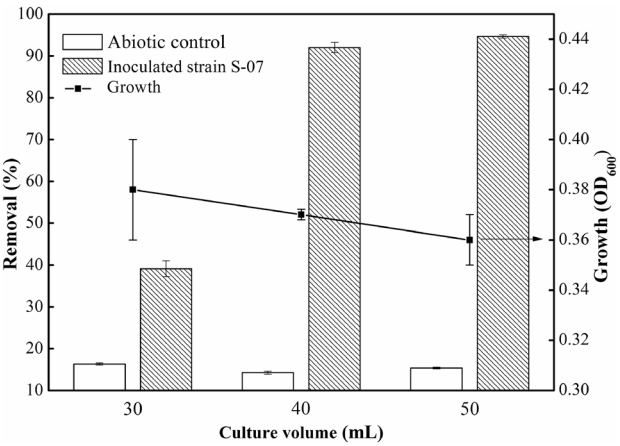

Fig. 5 Effect of culture volume on the growth of strain S-07 and the degradation of SM2 (70 °C, pH = 6.0, 50 rpm). Data are presented as the average value of triplicate measurements with standard deviation

Antibacterial activity analysis of SM2 and its metabolites

In this test, the supernatants from cultures of S-07 (E1) and the medium containing SM2 (E2) showed an obvious inhibition of *E. coli* K12 growth. The bacterial growth in the control (C) was considered to be 100%. The relative growth rates of E1 and E2 were 44.15 and 35.94%, respectively, suggesting that 55.85% of *E. coli* K12 growth in E1 and 64.06% in E2 were inhibited. As shown, the culture of strain S-07 growing in SM2-containing medium for 24 h at 70 °C retained little SM2 (<5%), suggesting that most of the SM2 was transformed to metabolites. This result revealed that the metabolites of SM2 retain high antimicrobial activity.

Degradation of other sulfonamides by strain S-07

SM1, SD and SMX can be degraded by strain S-07 after 24- and 48-h incubations. Among the three sulfonamides, strain S-07 showed the strongest removal of SM1. Nearly 72.96% of SM1 in medium was removed after 24 h, and 85.96% was removed after 48 h (Fig. 6).

Discussion

A thermophilic SM2-degrading bacterial strain (S-07) was obtained in this study. It was identified as *G. thermoleovorans* and is able to degrade SM2 at high temperature. The type strain of *G. thermoleovorans* also possesses the ability to degrade SM2, although at a much

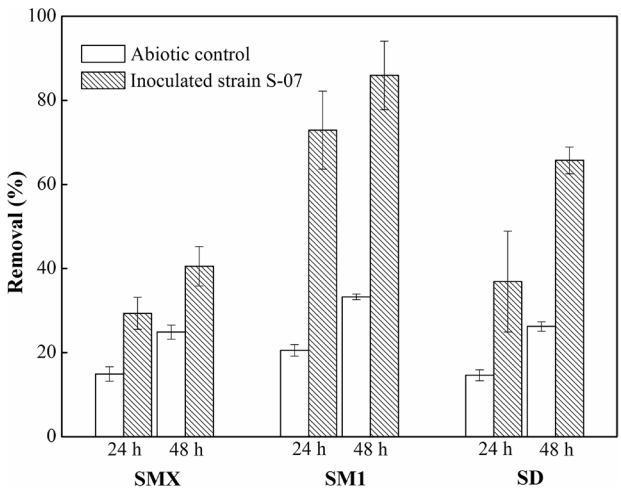

Fig. 6 Degradation of three other sulfonamides by strain S-07 (initial concentration of the sulfonamides: 10 mg/L, 70 °C, pH = 6.0, 50 rpm, 50 mL MMSM in a 100-mL Erlenmeyer flask). Data are presented as the average value of triplicate measurements with standard deviation

lower level than that of strain S-07. *Geobacillus* sp. have been reported to have the ability to degrade various organic compounds, including naphthalene, phenol, n-alkanes and organophosphonates (Kato et al. 2010; Obojska et al. 2002; Wang et al. 2006; DeFlaun et al. 2007). This is the first report of a thermophilic *Geobacillus* sp. strain performing sulfonamide biodegradation.

Temperature and the initial pH of the medium were important factors for the degradation of contaminants. SM2 decomposes at relatively high temperatures. Therefore, the degradation of the SM2 in the control experiments increased slightly with temperature. Moreover, temperature significantly affects pollutant biodegradation by affecting bacterial growth and some important enzymes during biodegradation (Peng et al. 2014). The pH value of the medium also affects the activity of enzymes in metabolic processes and changes the availability of nutrients in the growth environment. In addition, the pH of the medium affected the form of SM2 (anionic/cationic/non-ionized), which could further result in the difference of the degradation efficiency. In this study, degradation of SM2 by S-07 occurred at a high temperature and neutral pH, suggesting that this strain could be a good candidate for the degradation of sulfonamide residue in composting.

We then investigated the effect of rotation speed and culture volume on the degradation of SM2 by strain S-07 to evaluate the impact of oxygen content on the activity of the microorganism. The results showed there was no obvious difference in the SM2 degradation at rotation speeds of 0, 50, 100 rpm and culture volumes of 40 and 50 mL. However, when the rotation speed was increased to 150 rpm or the culture volume was decreased to 30 mL, the degradation of SM2 significantly decreased. This result might be caused by the strong shear stress resulting from the fast rotation speed and the low volume of the medium. Shearing changes the cellular metabolism (Philip et al. 2009), and the microorganism might primarily utilize available resources for growth rather than for the degradation of SM2. These results also indicated that oxygen content had a minor effect on SM2 transformation by strain S-07. Therefore, SM2 biodegradation by strain S-07 did not require the participation of oxygen. To verify this conclusion, complementary anaerobic experiments (Table S1) with an initial bacterial concentration of $OD_{600}=0.333$ were conducted to test the anaerobic degradation of SM2. After 24 h, there was no cell growth, but the removal of SM2 was similar to that observed in the aerobic experiment (the bacterial concentration was $OD_{600}=0.363$ after 24 h). This result demonstrates that, while strain S-07 needs oxygen to utilize the carbon source and other growth factors for cell proliferation, oxygen is not a limiting factor for the metabolism of SM2.

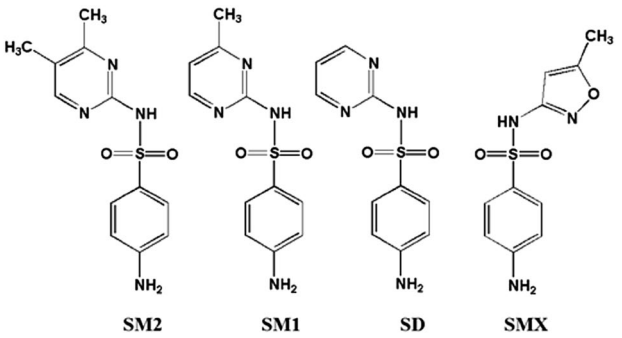

Fig. 7 Chemical structures of SM2, SM1, SD and SMX

More than 95% of SM2 was degraded by strain S-07 in 24 h at optimal conditions (70 °C, pH = 6.0, rotation speed: 50 rpm, 50 mL of MMSM in a 100-mL Erlenmeyer flask, 10 mg/L of SM2). Considering the results of the preliminary experiment (Figs. S1, S2) and the present study, strain S-07 degrades SM2 via co-metabolism, which is defined as the transformation of a non-growth substrate by growing cells in the presence of growth substrate, or by resting cells in the absence of a growth substrate (Criddle 1993). Microbial co-metabolism is an important strategy to eliminate recalcitrant pollutants, such as aromatic compounds, pesticides, petroleum hydrocarbons, halogenated hydrocarbons, and so on (Luo et al. 2014; Sharma et al. 2014; Li et al. 2015). Previous studies have shown that antibiotics can be degraded faster in the presence of more easily utilized substrates. The supply of nutrients, such as carbon sources, nitrogen sources and vitamins, could significantly improve the growth of microbial cells and the activity of key enzymes, further enhancing the capabilities of the co-metabolic system (Luo et al. 2014). In the preliminary experiment, MMSM was supplemented with yeast extract and glucose to provide growth factors and a carbon source for the microorganism. No growth and biodegradation were observed in MMSM without yeast extract and glucose. In medium containing glucose but without yeast extract, no growth was observed (Fig. S1). However, the biodegradation of SM2 was observed, which suggests that strain S-07 inoculation could lead to the degradation of SM2 and that glucose cannot facilitate the growth of microorganisms without other trace growth factors. In medium lacking glucose but containing some yeast extract, the microorganism showed weak growth, and SM2 was partly biodegraded (Fig. S2). Thus, strain S-07 mediated the biodegradation of SM2 via co-metabolism under conditions of easily available carbon sources and in the presence of other nutrients.

Strain S-07 showed the ability to degrade other sulfonamides, but this effect was dissimilar. The different degradation abilities among the different types of sulfonamides might reflect differences in the molecular structures of

drugs (Fig. 7), as well as their toxicity to microorganisms. There are many types of sulfonamide residue in manure compost and other environmental compartments. Therefore, it is necessary to assess the biodegradation of other sulfonamides by strain S-07. A previous study showed that the bacterium was capable of degrading one sulfonamide substance and might also degrade many other sulfonamides (Ingerslev and Halling-Sørensen 2000). Reis et al. (2014) used an SMX-degrading strain, PR1, to degrade other antimicrobial sulfonamides. Under certain conditions, strain PR1 degraded all of the drugs tested, and the degradation efficiencies were different. Müller et al. (2013) reported that the enzymes that require primary biodegradation are class-specific rather than compound-specific. The adapted microbial communities were able to biodegrade a set of structurally similar sulfonamides. The capacity of strain S-07 to degrade several types of sulfonamides reinforced the potential of this strain in compost bioremediation.

In the present study, the metabolites of SM2 retained high antibacterial activity, although their toxicities to microorganism slightly declined compared with SM2, which implies that SM2 is not completely degraded under certain conditions. At present, there are limited studies on the metabolites of SM2. A previous study by Topp et al. (2013) showed that *Microbacterium* sp. strain C448 mineralized SM2 and excreted stoichiometric amounts of 2-amino-4,6-dimethylpyrimidine, which is one type of raw material for the synthesis of SM2; it also possesses high toxicity. Thus, future approaches should adjust some of the experimental conditions, such as the culture time and temperature or the addition of other microorganisms, to completely degrade SM2.

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References

Batt AL, Bruce IB, Aga DS (2006a) Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. Environ Pollut 142:295–302

Batt AL, Snow DD, Aga DS (2006b) Occurrence of sulfonamide antimicrobials in private water wells in Washington County, Idaho, USA. Chemosphere 64:1963–1971

Boxall AB, Johnson P, Smith EJ, Sinclair CJ, Stutt E, Levy LS (2006) Uptake of veterinary medicines from soils into plants. J Agric Food Chem 54:2288–2297

Criddle CS (1993) The kinetics of cometabolism. Biotechnol Bioeng 41:1048–1056

DeFlaun MF, Fredrickson JK, Dong H, Pfiffner SM, Onstott TC, Balkwill DL, Streger SH, Stackebrandt E, Knoessen S, van Heerden E (2007) Isolation and characterization of a *Geobacillus thermoleovorans* strain from an ultra-deep South African gold mine. Syst Appl Microbiol 30:152–164

Dmitrienko SG, Kochuk EV, Apyari VV, Tolmacheva VV, Zolotov YA (2014) Recent advances in sample preparation techniques and methods of sulfonamides detection: a review. Anal Chim Acta 850:6–25

Dolliver H, Kumar K, Gupta S (2007) Sulfamethazine uptake by plants from manure-amended soil. J Environ Qual 36:1224–1230

Dolliver H, Gupta S, Noll S (2008) Antibiotic degradation during manure composting. J Environ Qual 37:1245–1253

Gao PP, Mao DQ, Luo Y, Wang LM, Xu BJ, Xu L (2012) Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. Water Res 46:2355–2364

García-Galán M, Silvia Díaz-Cruz M, Barceló D (2008) Identification and determination of metabolites and degradation products of sulfonamide antibiotics. TrAC Trends Anal Chem 27:1008–1022

Grote M, Vockel A, Schwarze D, Mehlich A, Freitag M (2004) Fate of antibiotics in food chain and environment originating from pigfattening (Part 1). Fresenius Environ Bull 13:1216–1224

Herzog B, Lemmer H, Horn H, Müller E (2013) Characterization of pure cultures isolated from sulfamethoxazole-acclimated activated sludge with respect to taxonomic identification and sulfamethoxazole biodegradation potential. BMC Microbiol 13:276

Heuer H, Schmitt H, Smalla K (2011) Antibiotic resistance gene spread due to manure application on agricultural fields. Curr Opin Microbiol 14:236–243

Hirsch R, Ternes T, Haberer K, Kratz KL (1999) Occurrence of antibiotics in the aquatic environment. Sci Total Environ 225:109–118

Hsu JT, Chen CY, Young CW, Chao WL, Li MH, Liu YH, Lin CM, Ying C (2014) Prevalence of sulfonamide-resistant bacteria, resistance genes and integron-associated horizontal gene transfer in natural water bodies and soils adjacent to a swine feedlot in northern Taiwan. J Hazard Mater 277:34–43

Ingerslev F, Halling-Sørensen B (2000) Biodegradability properties of sulfonamides in activated sludge. Environ Toxicol Chem 19:2467–2473

Jiang BC, Li A, Cui D, Cai R, Ma F, Wang YN (2014) Biodegradation and metabolic pathway of sulfamethoxazole by *Pseudomonas psychrophila* HA-4, a newly isolated cold-adapted sulfamethoxazole-degrading bacterium. Appl Microbiol Biotechnol 98:4671–4681

Kato T, Miyanaga A, Kanaya S, Morikawa M (2010) Gene cloning and characterization of an aldehyde dehydrogenase from long-chain alkane-degrading *Geobacillus thermoleovorans* B23. Extremophiles 14:33–39

Kim SD, Cho J, Kim IS, Vanderford BJ, Snyder SA (2007) Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water Res 41:1013–1021

Larcher S, Yargeau V (2011) Biodegradation of sulfamethoxazole by individual and mixed bacteria. Appl Microbiol Biotechnol 91:211–218

Li SS, Li DN, Yan W (2015) Cometabolism of methyl tert-butyl ether by a new microbial consortium ERS. Environ Sci Pollut Res 22:10196–10205

Luo W, Zhu XC, Chen WT, Duan ZB, Wang L, Zhou Y (2014) Mechanisms and strategies of microbial cometabolism in the degradation of organic compounds-chlorinated ethylenes as the model. Water Sci Technol 69:1971–1983

Mitchell SM, Ullman JL, Bary A, Cogger CG, Teel AL, Watts RJ (2015) Antibiotic degradation during thermophilic composting. Water Air Soil Pollut 226:1–12

Müller E, Schussler W, Horn H, Lemmer H (2013) Aerobic biodegradation of the sulfonamide antibiotic sulfamethoxazole by activated sludge applied as co-substrate and sole carbon and nitrogen source. Chemosphere 92:969–978

Obojska A, Ternan NG, Lejczak B, Kafarski P, McMullan G (2002) Organophosphonate utilization by the thermophile *Geobacillus caldoxylosilyticus* T20. Appl Environ Microbiol 68:2081–2084

Peng XX, Qu XD, Luo WS, Jia XS (2014) Co-metabolic degradation of tetrabromobisphenol A by novel strains of *Pseudomonas* sp. and *Streptococcus* sp. Bioresource Technol 169:271–276

Philip S, Sengupta S, Keshavarz T, Roy I (2009) Effect of impeller speed and pH on the production of poly (3-hydroxybutyrate) using *Bacillus* cereus SPV. Biomacromolecules 10:691–699

Ramaswamy J, Prasher SO, Patel RM, Hussain SA, Barrington SF (2010) The effect of composting on the degradation of a veterinary pharmaceutical. Bioresour Technol 101:2294–2299

Reis PJ, Reis AC, Ricken B, Kolvenbach BA, Manaia CM, Corvini PF, Nunes OC (2014) Biodegradation of sulfamethoxazole and other sulfonamides by *Achromobacter denitrificans* PR1. J Hazard Mater 280:741–749

Selvam A, Zhao ZY, Li YC, Chen YM, Leung K, Wong J (2013) Degradation of tetracycline and sulfadiazine during continuous thermophilic composting of pig manure and sawdust. Environ Technol 34:2433–2441

Sharma T, Rajor A, Toor AP (2014) Degradation of imidacloprid in liquid by *Enterobacter* sp. strain ATA1 using co-metabolism. Bioremediat J 18:227–235

Topp E, Chapman R, Devers-Lamrani M, Hartmann A, Marti R, Martin-Laurent F, Sabourin L, Scott A, Sumarah M (2013) Accelerated biodegradation of veterinary antibiotics in agricultural soil following long-term exposure, and isolation of a sulfamethazine-degrading sp. J Environ Qual 42:173–178

Van Dijk J, Keukens HJ (2000) The stability of some veterinary drugs and coccidiostats during composting and storage of laying hen and broiler faeces. In: Ginkel, LA, Ruiter, A (eds) Residues of veterinary drugs in food, Proceedings of the Euro Residue IV Conference, Veldhoven, 8–10 May 356–360

Wang L, Tang Y, Wang S, Liu RL, Liu MZ, Zhang Y, Liang FL, Feng L (2006) Isolation and characterization of a novel thermophilic *Bacillus* strain degrading long-chain n-alkanes. Extremophiles 10:347–356

Watts C, Crathorne B, Fielding M, Killops S (1982) Nonvolatile organic compounds in treated waters. Environ Health Perspect 46:87

Wu XF, Wei YS, Zheng JX, Zhao X, Zhong WK (2011) The behavior of tetracyclines and their degradation products during swine manure composting. Bioresour Technol 102:5924–5931

Zhang WW, Wen YY, Niu ZL, Yin K, Xu DX, Chen LX (2012) Isolation and characterization of sulfonamide-degrading bacteria *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51. World J Microbiol Biotechnol 28:447–452