Simultaneous High-Efficiency Removal of Sulfamethoxazole and zinc (II) From Livestock and Poultry Breeding Wastewater by a Novel Dual-Functional Bacterium, *Bacillus* sp. SDB4

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Abstract

The complex mixtures of antibiotics and heavy metals are commonly existed in livestock and poultry breeding wastewater. Effective and simultaneous removal of these toxic compounds by microorganisms, especially single strains remains a considerable challenge. In this study, a novel functional strain SDB4, isolated from duck manure and identified as *Bacillus* sp., has been shown to possess high removal abilities for both sulfamethoxazole (SMX) and Zn^{2+} . The maximum removal efficiency achieved 73.97% for SMX and 84.06% for Zn^{2+} within 48 h in sole system. It has great potential for eliminating SMX along with Zn^{2+} , 78.45% of SMX and 52.91% of Zn^{2+} were removed in the $20 \text{ mg}\cdot\text{L}^{-1}$ SMX and $100 \text{ mg}\cdot\text{L}^{-1}$ Zn^{2+} binary system. Furthermore, the SMX-biotransformation ability of SDB4 was enhanced at low concentrations of Zn^{2+} (below $100 \text{ mg}\cdot\text{L}^{-1}$). N^4 -acetyl-SMX was identified as the main stable transformation product during SMX bioremoval. FTIR analyses revealed that OH, NH_2 , C=O, C-N/N-H and C-O-C played major roles in the biosorption of Zn^{2+} . Our study of the dually functioning strain SDB4 provides a potential application for the simultaneous biological removal of antibiotics and heavy metals.

1. Introduction

With the dietary evolution of humans, the actual scale of global animal rearing and meat production has increased, resulting in intensification of livestock farming (Tullo et al. 2019). In contrast with traditional feeding methods, livestock diseases are increasingly complex in the intensive and large-scale feeding industry. These diseases spread rapidly and cause huge economic losses once outbreak becomes endemic. Consequently, antibiotics such as sulfonamides and tetracyclines, have been widely used to prevent livestock diseases (Shao et al. 2020). At the same time, heavy metals including zinc and copper have also been extensively used as feeding additives to increase production (Li et al. 2019). However, only partial antibiotics/heavy metals can be metabolized/absorbed by livestock, causing a large number of residues in the excreta (Liu et al. 2016; Xu et al. 2016). These elements are readily accessible to the land and easily entered the water through leaching and direct runoff, thereby spreading drug and heavy metal contamination (Lillenberg et al. 2010; Pan et al. 2018; Trenouth and Gharabaghi 2015). The commonly coexistence of antibiotics and heavy metals in high concentrations in livestock farming wastewater generate combined contamination (Riaz et al. 2020). Over time, high levels of complex contaminants also contribute to the accumulation of antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) (Chen et al. 2018; Christou et al. 2017). The compound pollution of antibiotics and heavy metals in aquatic environments has become an international problem, causing harm to human life and ecological health (He et al. 2017; Hou et al. 2019; Xing and Jin 2018). Therefore, it is urgently needed to remove the combined contaminants from aquaculture wastewater.

The removal of antibiotics and heavy metals from wastewater is a challenging issue, since the complicated characteristics of combined contamination. Different sorts of composite pollutants have complicated properties (hydrophobic/hydrophilic, negative/positive surface charge characteristics, etc.), which makes the treatment of wastewater even more difficult (Ma et al. 2014). Several physical-chemical

methods have been adopted to simultaneously remove antibiotics and heavy metals. Some studies of flocculants were investigated for their synergistic removal potential of antibiotics and heavy metals, and flocculation mechanisms of combined pollutants were proposed (Hou et al. 2019; Zhang et al. 2014). Antibiotics and heavy metals could be absorbed by some special materials. The capability of hybrid silicate-hydrochar composite to remove tetracycline, Cu^{2+} and Zn^{2+} was investigated (Deng et al. 2020). Shao et al. explored the synthesis and application of nanoscale zero-valent iron in simultaneous chlortetracycline and Cu^{2+} removal, the removal mechanisms of combined pollutants were also studied (Shao et al. 2020). Ma et al. indicated that ciprofloxacin and Cu^{2+} can be removed simultaneously by coadsorption using graphene hydrogels (Ma et al. 2020). However, traditional physical and chemical methods require high cost, spend a lot of material resources, and even introduce new pollutants, limiting the application. Microbial remediation, a competitor in environmental engineering due to its cost-effectiveness and environmental friendliness for simultaneous treatment of various chemical pollutants, could be an alternative to traditional chemical and physical technologies (Shah and Shah 2020; Verma and Kuila 2019). The bioremoval of antibiotics or heavy metals has been extensively studied in the literature (Chen and Xie 2018; Qin et al. 2020). Nevertheless, only a few articles have reported simultaneous bioremoval of antibiotics and heavy metals by microbial consortia in long time (12 days) (Al-Gheethi et al. 2015). Limited information was available about the removal performance of microorganisms, especially single strains in shorter time. Meanwhile, the differences of bioremoving mechanisms between antibiotics/heavy metals single and antibiotics-heavy metals binary systems are still unclear. Hence, it is essential to isolate pure bacterium with antibiotics and heavy metals simultaneous removal ability and study its further use in aquaculture wastewater microbial remediation.

In this study, SMX and Zn^{2+} were selected as models due to the intensive use in poultry feeding industry (Li et al. 2019; Shao et al. 2020). A novel aerobic strain SDB4, isolated from duck manure and identified as *Bacillus* sp. based on its physiological characteristics and 16S rRNA sequence, was applied for simultaneous removal of SMX and Zn^{2+} . The purposes of this study were to (1) isolate and identify a dual-functional strain from duck manure, (2) access the bioremoval characteristics of SMX or/and Zn^{2+} by strain SDB4 under single and binary systems, and (3) examine the bioremoval mechanisms of SMX and Zn^{2+} by strain SDB4. This study will provide theoretical basis for strain SDB4 in treating actual aquaculture wastewater.

2. Materials And Methods

2.1. Medium and chemicals

Luria-Bertani (LB) medium was prepared by mixing $10 \text{ g}\cdot\text{L}^{-1}$ tryptone, $5 \text{ g}\cdot\text{L}^{-1}$ yeast extract and $10 \text{ g}\cdot\text{L}^{-1}$ NaCl in 1000 mL deionized water, adjusted to 7.0 pH using NaOH. In total, the solid medium was prepared by adding 1.5-2.0% (w/v) agar. All media were autoclaved for 30 min at 121°C before use.

SMX was purchased from Aladdin Company with purity above 98% (China). Zinc sulfate heptahydrate and other reagents were all analytical grade purchased from Tianjin Damao Chemical Reagent Factory, China.

2.2. Screening SMX-Zn²⁺ resistant and bioremoving bacteria

In order to enrich SMX and Zn²⁺ resistant strain, which was isolated from the duck manure collected from a farm in Shandong, China, an aliquot of 10 g duck droppings was added to 100 mL of sterile growth LB medium containing 5 mg·L⁻¹ SMX and 5 mg·L⁻¹ Zn²⁺ in a 250 mL conical flask. After 5 days of incubation at 30°C and 150 rpm in the dark, 10 mL enrichment suspensions were transfer to 100 mL fresh LB containing 10 mg·L⁻¹ SMX and 10 mg·L⁻¹ Zn²⁺. The enrichment process was continued 9 times using the same methods, with both SMX and Zn²⁺ concentrations increased by 5mg·L⁻¹ during each inoculation period until the concentrations both reached 50 mg·L⁻¹ in LB. After 45 days, the concentrated suspensions were coated on LB agar plates and grown at 30°C in constant temperature and dark environment. Ten isolated colonies were extracted and purified by repeated streaking on the fresh LB agar plates. The purified strains with SMX and Zn²⁺ resistance were cultivated in fresh LB to test the removal efficiency of SMX/Zn. Finally, SDB4 showed the best SMX bioremoval ability.

2.3. Identification of dual-functional strain

According to the manufacturer's instructions, genomic DNA was extracted with the Bacterial Genome DNA Extraction Kit (Tiangen Biotech Co., Ltd.). The following primers were used to amplify the PCR of the gene encoding 16S rRNA: 27f and 1492r was the forward and reverse primer, respectively (Pan et al. 2018). The PCR products were sequenced in MajorBio (Shanghai, China).

The IDENTIFY tool was used for comparing the sequence with the available DNA sequences in EzTaxon (<http://www.ezbiocloud.net/eztaxon>). MEGA version 7.0 software was used to analyze the phylogenesis, and the phylogenetic tree was constructed with the neighbor-joining method.

2.4. Experiments design

In the single pollution system, to determine SMX/Zn²⁺ bioremoval capability, firstly, SDB4 was firstly grew in LB medium at 30°C and shaken with a speed of 150 rpm. The cells were harvested 24 hours later and centrifuged at 8000 rpm for 15 min at 4°C. After centrifugation, the SDB4 cells were then washed three times with 0.9% sterilized sodium chloride solution. The suspensions of SDB4 cell were finally transferred to sterilized LB liquid medium containing SMX/Zn²⁺ for the bioremoval of SMX/Zn²⁺. The bioremoval experiments were performed in a batch of 250 mL flasks. The samples were collected periodically for determining SMX/Zn²⁺ concentrations.

Harmful effects were caused by the accumulation and persistence of antibiotics in various environmental conditions, meanwhile heavy metals are commonly occur together with antibiotics in livestock and poultry wastewater (Shao et al. 2020; Zhang et al. 2017). So a wide range of environmental factor and

the effect of heavy metals on SMX bioremoval have been explored during the study. Based on the previous preliminary experiments of SMX bioremoval, four groups of experiments were designed. Various SMX concentrations ($20\text{--}800\text{ mg}\cdot\text{L}^{-1}$), pH ($4.0\text{--}10.0$), temperature ($15\text{--}40^{\circ}\text{C}$) and Zn^{2+} concentrations ($20\text{--}250\text{ mg}\cdot\text{L}^{-1}$).

The above four groups of experiments were all carried out under sterile conditions; the initial concentration of SMX was all $20\text{ mg}\cdot\text{L}^{-1}$ except the SMX concentration influence test; and all flasks were wrapped in aluminum foil to prevent SMX photodegradation.

Zn^{2+} bioremoval experiments were performed in flasks containing 100 mL LB liquid medium supplemented with single metal ($20\text{--}250\text{ mg}\cdot\text{L}^{-1}$).

In the binary pollution system, in order to investigate the removal efficiencies of complex pollutants by SDB4, the SMX- Zn^{2+} binary system in LB liquid medium consists of $20\text{ mg}\cdot\text{L}^{-1}$ SMX and $100\text{ mg}\cdot\text{L}^{-1}$ Zn^{2+} .

2.5 Analytical methods

The changes of bacterial concentration were monitored by a UV-1100 ultraviolet spectrophotometer (Mapada in China) at 600 nm and expressed as OD value.

Briefly, 2ml samples were extracted from each flask, and filtered through a 0.45 μm filter membrane to remove impurities and biomass, then stored at 4°C for further measurement. SMX was tested by high-performance liquid chromatography (HPLC) (Agilent 1200 Series). The determination conditions and the mobile phase composition were set as reported previously (Wang and Wang 2018).

The biotransformation metabolites of SMX were analyzed by Ultra-high-pressure liquid chromatography tandem mass spectrometry (UHPLC-MS-MS, Ultimate 3000/Q-Exactive, USA). Acquity® UPLC® BEH C18, $1.7\text{ }\mu\text{m}$, $50\times 2.1\text{ mm}$ column (Waters Corporation) was used. The flow rate was $600\text{ }\mu\text{L}\cdot\text{min}^{-1}$ and the volume of injection was 5 μL . The mobile phases were (A) water-methanol (95:5%, v/v) containing 0.1% acetic acid and (B) acetonitrile-methanol (50:50%, v/v) containing 0.1% acetic acid. The chromatographic analysis was performed in gradient mode, starting at 90% A, holding for 1 min, and then rising to 90% B within 6 min. The electrospray interface (ESI) was provided on the mass spectrometer, and the ESI source was operated in positive ionization mode with the ionspray voltage set for 4.0 kV at 300°C . Full scans were obtained from m/z 50–600 to identify the metabolites. Metabolites in single SMX system and SMX- Zn^{2+} binary system were identified by comparing with standard compounds.

The residual Zn^{2+} concentration in the solution was determined using the inductively coupled plasma optical emission spectrometry (ICP-OES) method.

Fourier transform infrared spectroscopy (FTIR) was used to analyze the changes of functional groups in control sample (without adding pollutants), $100\text{ mg}\cdot\text{L}^{-1}$ Zn^{2+} contaminated samples, $20\text{ mg}\cdot\text{L}^{-1}$ SMX

and 100 mg·L⁻¹ Zn²⁺ co-contaminated samples. The scanning range was 500–4000 cm⁻¹, resolution of 4 cm⁻¹, and scanning frequency of 32.

2.6 Statistical analysis

All the experiments were repeated three times and the results were averaged. The bioremoval efficiency (%) was calculated by the following equations:

$$\text{Bioremoval efficiency (\%)} = \frac{(C_0 - C_t) * 100}{C_0} \quad (1)$$

where C₀ (mg·L⁻¹) and C_t (mg·L⁻¹) are SMX or Zn²⁺ concentration in solution at initial and time t, respectively.

3. Results And Discussion

3.1. Isolation and identification of the dual-functional strain

The dual-functional strain SDB4, isolated from the duck manure, could grow on solid LB medium with both SMX and Zn²⁺. Strain SDB4 is an aerobic gram-negative strain, and waxy, circular, non-translucent colonies were formed on LB solid plate. The partial 16S rRNA gene sequence analysis of strain SDB4 (1426 bp, GenBank accession number: MT647568) showed a close relationship to the genus *Bacillus* and the similarity to *Bacillus paramycoides* (GenBank accession number MA0101000012, Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; *Bacillus*) was 98.59%.

Based on the morphological characteristics and phylogenetic analysis, SDB4 was identified as *Bacillus paramycoides*. According to the 16S rRNA gene sequences and other close phylogenetically relatives, the phylogenetic tree was constructed (Fig. 1). *Bacillus paramycoides* is a novel specie of the *Bacillus cereus* group, originally isolated from a diverse marine environment (Liu et al. 2017). There are few studies on its resistance to antibiotics/heavy metals. It is the first manuscript of a strain belonged to *Bacillus* sp., which could simultaneously bioremove SMX and Zn²⁺.

3.2. Bioremoval performance of SMX by strain SDB4

3.2.1. Effect of initial concentration on SMX bioremoval

Six initial SMX concentrations (20, 50, 100, 200, 400 and 800 mg·L⁻¹) were adopted to investigate the effect of SMX concentration on SMX bioremoval efficiency by strain SDB4. The inoculation dose was 10% (v/v), the initial pH was set as 7 and the temperature was adjusted to 30°C. When SDB4 was added to LB, the bacteria growth and SMX removal efficiency gradually decreased with the increase of SMX concentration. Figure 2a, b show the remained concentration ranged from 6 to 310 mg·L⁻¹ when the average initial concentration of SMX changed from 20 to 800 mg·L⁻¹, of which the SMX removal

efficiency within 48 hours was 72.96, 70.14, 68.38, 66.93, 64.85, and 61.62%, respectively. With an initial concentration of SMX is $20 \text{ mg}\cdot\text{L}^{-1}$, the SMX removal efficiency reached a maximum of 72.96%.

With the increasement of SMX initial concentration, the removal rate of SDB4 was enhance. Assessing the initial SMX concentration from 20 to $800 \text{ mg}\cdot\text{L}^{-1}$, the bioremoval rate of SDB4 in 48 hours was 0.31, 0.78, 1.55, 2.93, 5.48, and $10.38 \text{ mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$, respectively. Previous study indicated that some bacterium occurred very fast SMX bioremoval rate of $5.0 \text{ mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ (Wang and Wang 2018). Results in this study showed higher SMX bioremoval capacity compared with previous studies. SDB4 is capable of utilizing SMX as a co-metabolic carbon source, exhibit an excellent bioremoval ability in a wide range of initial SMX concentration and can withstand the selection pressure of $800 \text{ mg}\cdot\text{L}^{-1}$ SMX.

3.2.2. Effect of pH on SMX bioremoval

pH is an important factor affecting microbial physiology, the solubility and ionic state of organic pollutants, thereby affecting the ability of microorganisms to degrade organic pollutants (Wang and Wang 2018; Zhang et al. 2017). Figure 2c,d depict the effect of different initial pH on the bioremoval of SMX by strain SDB4. The effects of pH value on SMX removal and bacterial growth were investigated under the conditions of pH 4.0–10.0. When the pH was maintained at 4, 5, 6, 7, 8, 9 and 10, the removal efficiency obtained was 45.25, 47.88, 63.42, 73.97, 67.58, 65.02 and 51.60%, respectively, within 48 hours under conditions of $20 \text{ mg}\cdot\text{L}^{-1}$ initial concentration and 30°C temperature. The highest SMX removal efficiency for strain SDB4 was observed to be 73.97% at pH 7. Similar results have also been reported in other biodegradants such as *Achromobacter* sp. S-3 and *Acinetobacter* sp. W1 (Huang et al. 2012; Wang and Wang 2018). This behavior caused by pH might be due to the negative effects of certain extreme pH values on functional enzyme (Lin et al. 2014).

Compared with acidic environment (pH 4–6), neutral and alkaline environment (pH 7–10) were more conducive to the bioremoval of SMX. There are two pKa values for SMX. It is present in the form of cation when the pH is below 1.6; It exists as the form of anion at a pH higher than 5.7; and when the pH value is between 1.6 and 5.7, it is in the form of neutral. The anion form is more easily degraded than other two forms in the process of AOPs (Qi et al. 2014). Similar information is available for biodegradation of SMX. Wang's research showed that *Acinetobacter* sp. was able to degrade SMX better in neutral and alkaline environment than acidic environment (Wang and Wang 2018). Thus, these results indicate that SDB4 can remove SMX effectively in mildly alkaline (pH 7–10) media. Based on the results above, SMX was bioremoved by SDB4 and occurred at a wide pH range, indicating that this strain might have a good application prospect.

3.2.3. Effect of temperature on SMX bioremoval

The temperature also significantly affects the biological removal of harmful substances by organisms (Zhang et al. 2017). Temperature is capable to change the activity of microorganism and solubility of SMX greatly (Wang and Wang 2018). In general, the solubility of SMX increased with temperature. However, there is an optimum temperature for the microbial activity of certain microorganism. In this

study, SMX bioremoval efficiency by SDB4 were studied at different temperatures from 15 to 40°C. Figure 2e shows the decrease in SMX concentration over time at all test temperatures. As shown in Fig. 2f, the removal efficiency of SMX at 15, 20, 25, 30, 35 and 40°C was 26.37, 40.30, 47.43, 73.96, 66.74 and 41.47%, respectively. Lowest or highest temperature will inhibit the growth of bacteria, resulting in a decrease in removal efficiency. In addition, the bioremoval rate of SMX reached to higher values of 0.318 and 0.280 mg·L⁻¹·h⁻¹ at 30 and 35°C, respectively. Therefore, SDB4 is an effective SMX bioremoval bacterium, with optimal temperature between 30 and 35 °C.

In the actual environment, the dependence of the strain on the ambient temperature will affect the bioremoval ability of SMX, which may cause the bioremediation effect be lower than expected (Deng et al. 2014). Fortunately, the strain SDB4 was able to grow and remove SMX at all test temperatures, though the bioremoval efficiency was reduced at low or high temperature.

3.2.4. Effect of Zn²⁺ on SMX bioremoval

To investigate the effect of the coexistence of Zn²⁺ on SMX bioremoval, 20–200 mg·L⁻¹ Zn²⁺ were added to the SMX solution with initial concentration of 20 mg·L⁻¹. The effects of initial Zn²⁺ concentrations on SMX removal were estimated by the SMX bioremoval efficiencies as shown in Fig. 2g. It was obvious to notice that SMX removal efficiency varied with Zn²⁺ concentration. The presence of Zn²⁺ (under 100 mg·L⁻¹) provided a slight stimulating effect on SMX removal. Compared to single SMX pollution system, SDB4 showed a more outstanding SMX removal ability in SMX-Zn²⁺ combined pollution system, whereas a higher concentration (100–200 mg·L⁻¹) exerted inhibition influence. Meanwhile, the concentration of bacteria decreased with the increase of Zn²⁺ addition (Fig. 2h).

As the Zn²⁺ concentration increased from 20 to 100 mg·L⁻¹, the removal efficiency of SMX in the medium increased from 74.09–78.06%, which was higher compared to that without Zn²⁺ in 48 h reaction time. At low concentrations of Zn²⁺ coexistence, SMX removal efficiency could be enhanced by accelerating the transportation of SMX from extracellular solution to inside cells and the formation of enzyme-metal-substrate complexes. Zn²⁺ accelerated SMX removal through uptaking more pollutants inside cells, meanwhile it might work as a cofactor for protein binding and be required for protein biological activity (Tang et al. 2016). The SMX removal efficiency decreased to 51.14 and 41.74% at 150 and 200 mg·L⁻¹ of Zn²⁺ concentration addition, it might be attributed to the inhibiting effect of Zn²⁺ over strain SDB4 resulting in lower removal efficiencies of SMX, Zn²⁺ may inhibit the activity of enzymes by combining with the sulfhydryl groups of the proteins. Additionally, superabundant Zn²⁺ competes with macronutrients (Mg²⁺, Ca²⁺ etc.), which are commonly used as cofactors for the formation of enzyme-metal-substrate complexes (Sherameti and Varma 2010).

3.3. Bioremoval performance of Zn²⁺ by strain SDB4

The effect of initial concentration on Zn²⁺ bioremoval by SDB4 was investigated under the temperature of 30°C and pH of 7. Figure 3a, b shows the Zn²⁺ bioremoval efficiency decreased with the increase of

initial Zn^{2+} concentration. As the initial concentration of Zn^{2+} was $20 \text{ mg}\cdot\text{L}^{-1}$, the Zn^{2+} removal efficiency and strain biomass reached the maximum, which were 84.06% and 16.51, respectively. The removal efficiencies were all above 70% with the initial Zn^{2+} concentration increased from $20 \text{ mg}\cdot\text{L}^{-1}$ to $150 \text{ mg}\cdot\text{L}^{-1}$, while the bacterial growth were decreased. However, the Zn^{2+} removal efficiencies decreased sharply to 67.40% and 55.36% when the initial Zn^{2+} concentration increased to $200 \text{ mg}\cdot\text{L}^{-1}$ and $250 \text{ mg}\cdot\text{L}^{-1}$, respectively. The strain biomass slowly grow of OD_{600} 10.03 and 7.98. This result may be due to high initial metal ion concentration leading to heavy metal toxicity that inhibits bacterial growth. The rapid decline in removal efficiency may be due to the lack of sufficient free radicals for metal microbial adsorption. It is possible that all metal ions can have good interaction with the binding sites at lower concentrations, resulting in higher removal efficiencies of Zn^{2+} . Similar conclusions have been summarized by other researchers (Jin et al. 2013; Xu et al. 2019). Therefore, the removal efficiency of SDB4 as adsorbent treating low concentration zinc pollution is relatively better, and we can adjust the injection amount of SDB4 based on the concentration of pollutants in the actual wastewater.

3.4. Simultaneous bioremoval SMX and Zn^{2+} by strain SDB4

In order to explore the interaction between SMX and Zn^{2+} bioremoval, the simultaneous removal by SDB4 were conducted in a binary system under the conditions of initial SMX concentration $20 \text{ mg}\cdot\text{L}^{-1}$ with adding $100 \text{ mg}\cdot\text{L}^{-1}$ of Zn^{2+} . The results are shown in Fig. 4 (a,b,c). Within 0–6 h, SDB4 started to fit the medium environment. With OD_{600} increasing from 1.95 to 6.86 during 6–18 h, organism biomass displayed an exponential phase. However, the cell started to grow slowly in the stationary period (18–48 h), and the reduction of carbon substrate may be the reason for this phenomenon.

In the process of simultaneous removal of SMX and Zn^{2+} by SDB4, the concentration of SMX and Zn^{2+} both decreased while the OD_{600} increased. As the organic carbon sources coexisted with SMX, the initial concentration of SMX dropped from $18.98 \text{ mg}\cdot\text{L}^{-1}$ to $4.09 \text{ mg}\cdot\text{L}^{-1}$, while the removal efficiency was 78.45% within 48h. The concentration of Zn^{2+} was decreased from $97.47 \text{ mg}\cdot\text{L}^{-1}$ to $45.90 \text{ mg}\cdot\text{L}^{-1}$ with the removal efficiency of 52.91% in 48 h. During the simultaneous removal of SMX and Zn^{2+} , The corresponding removal rates of SMX and Zn^{2+} were $0.310 \text{ mg}\cdot\text{L}^{-1}\cdot\text{h}$ and $1.074 \text{ mg}\cdot\text{L}^{-1}\cdot\text{h}$, respectively.

The relationship between SMX transformation and Zn^{2+} removal effect was analyzed through comprehensive analysis: First, SMX was preferentially bioremoved by SDB4 as a carbon source. Meanwhile, the concentration of Zn^{2+} was slightly removed in the first 6 h. SDB4 can rapidly remove SMX and a small amount of Zn^{2+} in the adaptive period, which indicates that SDB4 can adapt to the new liquid culture environment quickly. Compared with other strains, SDB4 has a short adaptation period (Su et al. 2019). Secondly, the decline of SMX concentration and the exponential increase of OD_{600} promoted Zn^{2+} to be removed at a high rate in the 6–18 h period, while SMX was also rapidly removed. These results show that strain SDB4 can adapt to SMX and Zn^{2+} combined pollution environment well, meanwhile it grow at high speed in the complex contaminants system. The biomass reached a high level, which could

remove the compound pollutants effectively. Both SMX and Zn^{2+} can be removed at a faster rate in the biomass logarithmic growth period, indicating that high concentration of biomass was able to improve the efficiency of various biochemical reactions. Dong has obtained similar conclusion (Dong and Hu 2021). Thirdly, when the SMX and Zn^{2+} concentration reduced to about $4 \text{ mg}\cdot\text{L}^{-1}$ and $46 \text{ mg}\cdot\text{L}^{-1}$, respectively. The cells of SDB4 started increase slowly, the SMX and Zn^{2+} removal rate became slower and the removal reaction reached balance. Consistent with the study of Shao et al., when the biomass of SDB4 increased slowly in the stationary period, the pollutants were no longer removed rapidly (Shao et al. 2018). Consequently, compared to the strains in previous research, SDB4 can not only remove SMX effectively, but also has excellent performance in removing SMX and Zn^{2+} simultaneously (Wang and Wang 2018).

3.5. Possible removal mechanisms

3.5.1 SMX biotransformation pathway

The main mechanism of SMX bioremoval was considered to be through biotransformation. The possible biotransformation metabolites of SMX in single and binary system were explored for further clarification of SMX bioremoval process. An intermediate product of SMX biotransformation was found in both systems. The intermediates were identified by detection and analysis of samples at 48 h with UHPLC-MS-MS. Extracted ion chromatogram (EIC) in the full scan mode indicated that the retention time of the intermediate was 3.46 min and 3.45 min, respectively (Fig. 5a, c). The peak area of binary system was larger than that of sole system, corresponding to low concentration coexisting Zn^{2+} promoting SMX bioremoval. The MS-MS full-scan spectrums of the intermediates were acquired in the positive ion mode (Fig. 5b, d). Both dominant ion peaks of the intermediate were at m/z 134 and 198, which were consistent with N^4 -acetyl-SMX (Ac-SMX), the transformation products of SMX.

Biodegradation and biotransformation are expected to be effective ways for eliminating SMX (Yang et al. 2016). Previous study indicated that N-hydroxy-SMX and N^4 -acetyl-SMX represented the main stable metabolites in SMX biotransformation process when the antibiotic was used as the carbon source (Zhang et al. 2016). HO-SMX is more stubborn than SMX in the environment. Ac-SMX is less toxic than the parent compound, meanwhile acetylation is an important way to eliminate many therapeutic drugs (Reis et al. 2018). In this study, only one product N^4 -acetyl-SMX was detected. According to the structure of metabolite compound, arylamine N-acetyltransferases might be involved in the biotransformation of SMX by SDB4. As demonstrated in the previous study, SMX could be transformed into Ac-SMX by arylamine N-acetyltransferase (Zhang et al. 2016). The functional arylamine N-acetyltransferase enzyme BanatC encoded from gene *banatC* in the pathogen *Bacillus anthracis*, was found able to acetylate the sulfonamide antibiotic SMX (Pluvinaige et al. 2007). The arylamine N-acetyltransferases Nat-a and Nat-b, which purified and characterized from *Bacillus cereus* 10-L-2, was able to convert various aniline compounds into corresponding acetanilides and played a role in detoxification (Takenaka et al. 2009). In the process of SMX biotransformation by SDB4, a metabolite N^4 -acetyl-SMX was found, whose toxicity

was around 10% of SMX (Majewsky et al. 2014). This pathway could detoxify SMX effectively and environmentally friendly.

3.5.2 FTIR analysis

FTIR analysis was carried out to determine which functional groups of SDB4 were involved in Zn^{2+} adsorption. The uptake of heavy metal ions by functional groups will lead to higher or lower frequency shift of the spectrum (Rahim et al. 2020). The spectra in Fig. 6 shows that the band intensities of different regions have changed after the cells were contacted with single Zn^{2+} and SMX- Zn^{2+} combined pollutants. The peak value corresponded to OH moved from 3294 cm^{-1} to 3298 cm^{-1} after zinc biosorption in both systems. The peaks observed at $1630\text{--}1680\text{ cm}^{-1}$ corresponded to $\text{C}=\text{O}$ in amide I band, while the at $1530\text{--}1630\text{ cm}^{-1}$ were for C-N/N-H component of amide II in amide II band. The red shifts of amide bands I and II show that $\text{C}=\text{O}$, C-N/N-H play significant roles in the biosorption of Zn^{2+} , which is consistent with the results obtained by other researchers (Li et al. 2019). The polysaccharide groups were determined by vibrations at $1050\text{--}1090\text{ cm}^{-1}$, the red shift (from 1071 cm^{-1} to 1051 cm^{-1} and 1054 cm^{-1} , respectively) indicates that C-O-C is also involved in Zn^{2+} biosorption. In the sole and binary pollution systems, the disappearance of characteristic peaks related to hydroxyl groups on carboxylic acid suggests that OH participated in Zn^{2+} adsorption. The bending at $600\text{--}750\text{ cm}^{-1}$ represented amino groups, the shift and disappearance of amino group peaks, indicating the interaction of NH_2 in Zn^{2+} bioaccumulation. All the above implied the hydroxyl, amino, amide and polysaccharides played important roles in the biosorption of Zn^{2+} by SDB4.

In conclusion, SDB4 can not only biotransform SMX/biosorp Zn^{2+} in the sole pollution system, but also remove the two pollutants simultaneously in the binary pollution system. We proposed the mechanism of simultaneous removal of SMX and Zn^{2+} by SDB4 (Fig. 7).

4. Conclusions

A simultaneous SMX biotransforming and Zn^{2+} bioremoving *Bacillus* sp. strain SDB4, was isolated from duck manure in a farm, Shandong Province, China. In the single system, the strain showed effective bioremoval performance to SMX and Zn^{2+} , respectively. In the SMX- Zn^{2+} binary system, $20\text{ mg}\cdot\text{L}^{-1}$ SMX and $100\text{ mg}\cdot\text{L}^{-1}$ Zn^{2+} were removed simultaneously by strain SDB4 in 48 h, with SMX biotransformation rate of $0.310\text{ mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ and Zn^{2+} removal rate of $1.074\text{ mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$. The bioremoval ability of SMX was slightly improved at lower Zn^{2+} concentration ($< 100\text{ mg}\cdot\text{L}^{-1}$) as a result of Zn^{2+} accelerated the transportation of SMX from extracellular solution to inside cells during the reaction, but dropped with increase of Zn^{2+} concentration ($> 100\text{ mg}\cdot\text{L}^{-1}$). Furthermore, the bioremoval mechanisms were investigated by UHPLC-MS-MS, strain SDB4 possessed the same biotransformation route of SMX in both SMX single and SMX- Zn^{2+} binary pollutants removal systems. The functional groups of , NH_2 , $\text{C}=\text{O}$, C-N/N-H and C-O-C were mainly involved in Zn^{2+} bioremoval. This study indicates that *Bacillus* sp. strain

SDB4 has an important potential for microbial remediation of SMX and Zn^{2+} contaminants and the applied experiments in practical complex polluted wastewater treatment will be carried out.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: Not applicable.

Competing interests: The authors declare no competing interests.

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Authors' contributions Xi Chen: conceptualization, methodology, writing-original draft, investigation, formal analysis, data curation; Hai Lin: conceptualization, supervision, validation, resources, writing-review&editing, funding acquisition, project administration; Yingbo Dong: conceptualization, methodology, supervision, writing-review&editing, funding acquisition; Bing Li: investigation, conceptualization; Tingting Yin: formal analysis; Chenjing Liu: writing-review&editing.

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Figures

Figure 1

Phylogenetic tree based on the 16S rDNA gene, indicating the relationships between strain *Bacillus* sp. SDB4 and associated strains.

Figure 2

Effects of initial SMX concentration (a, b), pH (c, d), temperature (e, f) and coexisted Zn^{2+} concentration (g, h) on SMX bioremoval.

Figure 3

Effect of inoculation time on the bioremoval efficiency of Zn²⁺ (a) and OD₆₀₀(b).

Figure 4

Changes of SMX concentration and removal efficiency (a), changes of Zn²⁺ concentration and removal efficiency (b), changes of OD₆₀₀ (c) in the SMX-Zn²⁺ binary system.

Figure 5

Extracted ion chromatograms (EIC) and tandem mass spectrum of SMX biotransformation products. EIC of the 48 h sample of SMX biotransformation (a) and tandem mass spectrum of the intermediate product of SMX biotransformation (b) in sole system; EIC of the 48 h sample of SMX biotransformation (c) and tandem mass spectrum of the intermediate product of SMX biotransformation (d) in binary system.

Figure 6

FTIR spectra of strain SDB4 before (a), after (b) Zn²⁺ bioremoval and simultaneous bioremoval of SMX and Zn²⁺ (c).

Figure 7

Possible pathway for simultaneous biotransformation of SMX and biosorption of Zn²⁺ by strain SDB4.