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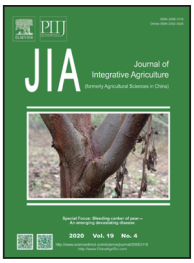

RESEARCH ARTICLE

Screening and degradation characteristics of a tylosin-degrading strain

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Abstract

Antibiotics residues have been accumulating in the environment day by day due to overuse of antibiotics. Recalcitrant antibiotic residues, such as tylosin (TYL), can cause serious environmental problems, which makes it important to eliminate TYL from the environment. It is important to eliminate TYL from the environment. In this study, a strain was isolated and purified from fermentation by-product that came from a TYL production factory. The TYL degrading strain was identified by its morphology, physiological and biochemical reactions and sequencing the PCR-amplified fragments of its 16S rDNA-coding genes. The temperature, shaking speed, initial TYL concentration, pH and inoculum sizes were investigated under simulated conditions by using single factor tests. The results showed that TYL2, a high efficient strain was isolated and was identified as *Brevibacillus borstelensis*. The degradation rate of TYL by this strain could reach to 75% with an initial concentration of 25 mg L^{-1} within 7 days under conditions of 7% *B. borstelensis* (v/v, 2×10^8 CFU mL^{-1}) at pH 7.0 and at 35°C. It is interesting that this strain has a very strong ability to degrade the TYL in natural sewage with the degradation rate of 65% within 7 days. This result could be helpful for the degradation of TYL and provide guidance for the degradation of other antibiotics.

Keywords: antibiotics, tylosin, screening, degrading strain, *B. borstelensis*, degradation characteristic

1. Introduction

Antibiotics have been developed and used in various fields, especially in the disease treatment and poultry breeding industry in recent decades. Due to the excessive use of these antibiotics, most administered antibiotics are not completely absorbed by the animal, and up to 67% of it is discharged into the environment in the form of parent drug and metabolites (Chee-Sanford *et al.* 2009). The residual of antibiotics could enter into the soil, water and other

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environments through various channels (Mompelat *et al.* 2009). The environmental pollution caused by antibiotics in aquatic systems and soil has received increasing concerns in the world (Selvam *et al.* 2012; Pan and Chu 2016).

Tylosin (TYL) was a sixteen-membered-ring macrolide antibiotic naturally produced by *Streptomyces fradiae* (Ma *et al.* 2015). It was widely used in livestock and poultry industry, as a livestock-specific antibiotic additives or veterinary treatment preparations to promote animal growth and protect them from illness (Prats *et al.* 2001). It has been found that the TYL residues widely exist in different environments. For example, the residues ranged from 0.015–1.7 mg L^{-1} in manure slurries; and were 1.2 μg L^{-1} in soil leachate; 6.0 μg L^{-1} in runoff and 0.3 μg g^{-1} in the swine manures (Kolz *et al.* 2005; Dolliver and Gupta 2008; Hoese *et al.* 2009). The residues could induce and promote the generation and spread of the related antibiotic resistance genes (ARGs) in different environments (Rodriguez *et al.* 2015; Chen *et al.* 2016; He *et al.* 2016), which may cause serious environmental and human health problem. Therefore, it is particularly important to effectively degrade and remove TYL from environments, especially aquatic environment because of its mobility.

The residues of TYL in environments could be degraded or removed through adsorption (Yin *et al.* 2018, 2019), hydrolysis and photolysis (Homen and Santos 2011; Sanscartier *et al.* 2011). In abiotic degradation methods, the UV/TiO_2 photocatalytic method could efficiently remove TYL about 97% in less than 60 min (Tassalit *et al.* 2011) and the UV/nano Ag/S_2 process also could remove TYL effectively (Saeideh *et al.* 2014). Guo *et al.* (2018) reported that maize straw coated with sulfide could enhance the sorption ability and the amount of TYL sorption on adsorbents reached approximately 80% of the total in 2.5 h. Mitchell *et al.* (2015) observed that TYL could be completely hydrolysed in acidic condition, but the degradation products of TYL may remain bioactive. However, these abiotic methods cannot completely degrade TYL and often incur high costs because of the complex structure of TYL. Therefore, a better method for removal should be identified and studied. Biodegradation has been considered as a method with strong specificity, low cost and secondary pollution, especially for TYL (Maki *et al.* 2006). It has also been found that TYL could be degraded by microorganisms (Flemming and Bent 2001). However, the biodegradation of TYL depends on not only the microorganisms that are responsible for TYL degrading but also the adaptable environmental factors (Liu *et al.* 2016; Zhang *et al.* 2018). Furthermore, few studies applied the degrading microorganism to the environment or focused on the treatment of practical application. Therefore, in the present study we intend to isolate and identify a degrading strain from TYL pharmaceutical waste and investigated its degradation characteristics, and on this basis, to investigate its ability to remove the TYL residues in the sewage water. In this way we expect to lay a foundation for further exploring the degradation mechanism, metabolic pathway and application of TYL, and to provide theoretical guidance for the future treatment of TYL in livestock, poultry breeding and pharmaceutical production.

2. Materials and methods

2.1. Materials

TYL standard (purity, 99.99%) was purchased from Dr. Ehrenstorfer, GmbH Company (Germany). Chromatographic grade methanol and acetonitrile (AN) were purchased from Fisher Scientific (Massachusetts, USA). Formic acid was used with superior grade reagent. Ultrapure water was produced by the Milli-Q Advantage A10 System from Millipore (Massachusetts, USA). All other chemicals used in the present research were analytical grade. The solutions used in this experiment were prepared as follows:

The phosphate buffered solution (0.1 mol L^{-1}): make the mixture of 615.0 mL of 0.1 mol L^{-1} K_2HPO_4 solution and 385.0 mL of 0.1 mol L^{-1} KH_2PO_4 solution to obtain 1 L of phosphate buffer solution with the pH value of 7.0.

TYL stock solution: dissolve 50.0 mg of TYL standard with methanol and make the final concentration as 5 mg L^{-1}.

TYL standard solution: the stock solution (5 mg L^{-1} TYL) was diluted with methanol to final concentrations of 12.5, 25.0, 50.0, 100.0, and 200.0 mg L^{-1}, respectively. A standard curve was generated with standard solution of different TYL concentrations. All the solutions were stored refrigerated at 4°C.

2.2. Isolation and identification of bacteria strain

Isolation of TYL-degrading bacteria strain Adding 10.0 g of the TYL fermentation by-product obtained from a TYL production factory to 90.0 mL of 0.1 mol L^{-1} phosphate buffered solution and were agitated on shaker at 30°C for 2 h. The supernatant was then aseptically serially diluted, down to 10^{-7}. Then, 10 μL of the supernatants from the 10^{-5}, 10^{-6} and 10^{-7} dilutions were spread over mineral salts medium (NH_4Cl 1 g, NaCl 1 g, K_2HPO_4 1.5 g, KH_2PO_4 0.5 g, $MgSO_4$ 0.2 g, agar 20 g, and sterile ultrapure water 1 L) with 50 mg L^{-1} of TYL as the only carbon source. These supernatant dilutions were then cultured for 3 days in an incubator at 30°C. During the screening process, the concentration of TYL was sequentially increased from 50 mg L^{-1} to 200 mg L^{-1}. Then, after incubation, individual colonies were picked from the plates and spread over peptone solid medium (peptone 5 g, NaCl 5 g, beef extract 3 g, agar 20 g

and sterile ultrapure water 1 L) until the single bacterium was isolated.

Identification of the bacteria strain DNA of the target bacteria was extracted using the DNA Rapid Extraction Kit (AXYGEN, USA). Extracted DNA was amplified by PCR using universal bacteria primer set: 27F: 5′-AGAGTTTGATCMTGGCTCAG-3′, and reverse to 1492R: 5′-TACGGYTACCTTG TTACGACTT-3′ (Poter and Hay 2007). PCR reaction mix: 5.0 μL of 10× *Ex Taq* buffer, 4.0 μL of 2.5 mmol L^{-1} dNTP Mix, 2.0 μL of 10 pmol L^{-1} primer 1, 2.0 μL of 10 pmol L^{-1} primer 2, 0.5 μL of 5 U *Ex Taq*, 2.0 μL of template, 36.5 μL of ddH_2O.

PCR amplification reaction conditions: Pre-denaturation at 94°C for 3.0 min, 1.0 cycle; annealing at 54°C for 30 s; extension at 72°C for 30 s, 24 cycles. The PCR products were stored at 4.0°C. PCR products were sequenced by Beijing Majorbio Sanger Bio-Pharm Technology Co., Ltd. The returned sequence was compared with the nucleic acid data in GenBank by BLAST Program.

The purified TYL-degrading bacteria was further inoculated on beef paste peptone solid medium and cultured at 35°C for 72 h. And then it was stained by Gram stain and the morphology identification was conducted by observing the colony shape, size, color and surface features using oil immersion microscopy (Huang *et al.* 2009; Wang *et al.* 2014). The physiological and biochemical characteristics of the TYL-degrading bacteria were investigated by the serological identification experiments. In addition, to understand the growth characteristics of the TYL-degrading bacteria, the TYL-degrading bacteria strain was inoculated into the beef extract peptone medium and was cultured at 30°C, with the shaking speed of 150 r min^{-1}. During the cultural period, the values of OD_{600} were determined every two hours by spectrophotometry (SP).

2.3. Determination of TYL concentration

The TYL concentration of the cultural solution was determined according to the method described by previous studies (Prats *et al.* 2001; Ma *et al.* 2015). A total of 5 mL of the sample was taken and added to mix solution consisting of 8.0 mL of AN and 8.0 mL of *n*-hexanes, then was further mixed with vortex for 1 min, and extracted by ultrasonic for 3 min, and was followed by centrifugation at the speed of 5000 r min^{-1} for 10 min. Then, the supernatant was filtered through a 0.22-μm pore size membrane. The concentration of TYL of the supernatant was determined by HPLC (Waters, USA). Chromatographic conditions are as follows: chromatographic column C18 (150 mm×4.6 mm, 3.5 mm, Waters, USA), the mobile phase of 0.1% formic acid water solution (A):AN (B)=70:30, the flow rate was 1.0 mL min^{-1}, the column temperature was 40°C. Ultraviolet detection wavelength was 285 nm, the sample injection volume was 10 μL. The method showed a good linearity within the range of 12.5–200.0 mg L^{-1} with a correlation coefficient of 0.9996. The recoveries at the concentration of 25.0–200.0 mg L^{-1} were more than 85%.

2.4. Experimental degradation of TYL

Degradation of TYL by the isolated bacteria strain The bacterial culture medium was spread plated on the mineral salts medium containing TYL, at increasing concentrations between 25–100 mg L^{-1}, as the sole carbon source. All the treatments were performed in triplicate. The medium was incubated in constant temperature incubator at 30°C for 3 days. Discrete colonies were picked from the plates and streaked out to provide pure single strains. A purified single strain was activated and inoculated with 2×10^8 CFU mL^{-1} into 100 mL liquid medium with 50 mg L^{-1} of TYL in 250-mL Erlenmeyer flask. The mixtures were cultured at 30°C, with the shaking speed of 150 r min^{-1} for 7 days and were sampled on 1, 3, 5, and 7 days after incubation for TYL determination. In the study, an uninoculated medium was used as the control treatment with triplicate. The degradation rate (D) was calculated using the following equations:

$$D\ (\%)=\{[(T_0-T_n)-(C_0-C_n)]/[(T_0-(C_0-C_n)]\}\times100$$

where D is the degradation rate of TYL; T_0 is the initial concentration of tylosin in the experimental group; T_n is the concentration of TYL in the experimental group at the *n* days; C_0 is the initial concentration of TYL in the control group; C_n is the concentration of TYL in the control group at the *n* days.

Optimization of strain degradation conditions Single factor tests were applied to determine the optimize culture conditions for TYL removal by degrading strain. The parameters including temperature (25–45°C), shaking speed (90–200 r min^{-1}), pH (5.0–11.0), the initial TYL concentration (25–150 mg L^{-1}) and the inoculum sizes (2×10^8–2×10^9 CFU mL^{-1}) were tested sequentially. The optimized parameter was used when the next factor was tested. For example, firstly, to obtain the best temperature for TYL-degrading strain 2 (TYL2) to degrade TYL, the liquid medium (pH=7) with 50 mg L^{-1} of TYL in 250-mL Erlenmeyer flasks were inoculated with 2×10^8 CFU mL^{-1} of TYL2 and were cultured at 150 r min^{-1} in oscillating incubator at different temperature (25, 30, 35, 40 and 45°C). Then shaking speed was optimized at the optimal temperature and other parameters remained unchanged. Other factors were tested in a similar way. Each treatment was performed in triplicate.

Degradation of TYL in sewage sample The highly efficient degradation strain was inoculated into 100-mL sewage samples and mineral medium in 250-mL Erlenmeyer flasks with 25 mg L^{-1} of TYL, and cultured for 7 days to investigate

the ability and efficiency of the TYL-degrading bacteria strain in sewage.

2.5. Data analysis and statistics

The figures were created by Origin 8.5. Statistical significance was assessed using one-way analysis of variance (ANOVA) and individual different was determined by Duncan's multiple range test. Probability levels of less than 0.05 had statistical significance (P<0.05).

3. Results and discussion

3.1. Isolation and identification of the TYL-degrading bacteria strains

Isolation and verification of the TYL-degrading bacteria strains Eight strains were isolated from TYL fermentation by-product of production factory. Four strains, named as TYL1, TYL2, TYL3, and TYL4 were found to be able to grow in mineral salts medium with TYL as the only carbon source, indicating that they were capable of degrading TYL. The degradation effects of four strains on TYL are shown in Fig. 1. The degradation rates of TYL at all treatments increased with time except for the control treatment. In the control group, no strain was added, so the degradation rate was not obvious within 7 days. The highest TYL degradation rates were observed after 7 days. The degradation rates of TYL by four strains TYL1, TYL2, TYL3, and TYL4 on the 7th day were 12.36, 46.15, 6.35, and 7.96%, respectively. Compared with degradation rates of TYL by the four strains on the first day, the degradation rates were increased by 4.61, 32.87, 5.35 and 6.35% for TYL1, TYL2, TYL3, and TYL4 respectively. It is very interesting that the significant differences in degradation effects on TYL existed among four bacteria strains. The bacteria strain named TYL2 had the strongest effects on the TYL degradation among all four strains. For example, the degradation rate of TYL by TYL2 was 46%, which was significantly higher (P<0.05) than the corresponding data of TYL1, TYL3 and TYL4, respectively. It indicates that TYL like other antibiotics such as oxytetracycline and gentamicin can be degraded by microorganisms in environments (Migliore *et al.* 2012; Liu *et al.* 2017). TYL belongs to the macrolide antibiotics which is difficult to degrade in environments compared to other antibiotics such as amino glycans. Therefore, the results of the present paper may provide the new way to remove the macrolide antibiotics residues from the environments.

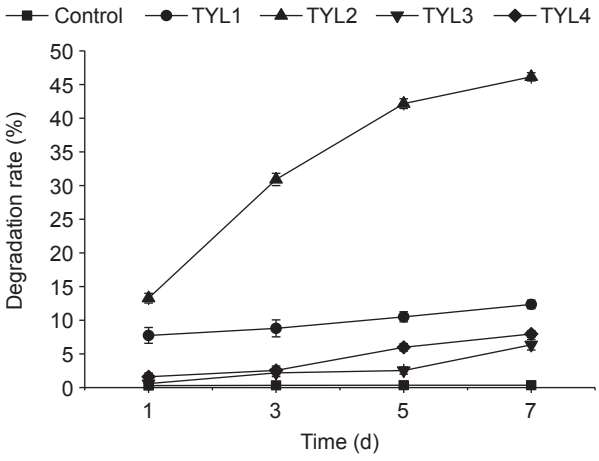

Fig. 1 The degradation rates of tylosin (TYL) by four TYL-degrading strains 1–4 (TYL1, TYL2, TYL3 and TYL4). The mean values and standard deviations (error bars) are presented (n=3).

Identification of the bacteria strain TYL2 The colony morphology of TYL2 is round, smooth and opaque milky white colonies 0.2–0.4 cm in diameter as shown in Fig. 2. The selected physiological and biochemical characteristics of strain TYL2 are shown in Table 1.

The gene sequence of TYL2 was submitted to National Center for Biotechnology Information (NCBI) for comparative analysis of nucleic acid data in GenBank by basic local alignment search tool (BLAST) Program. The result showed that the 16S rDNA gene sequence of TYL2

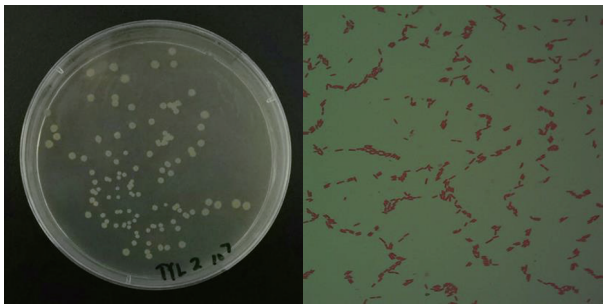

Fig. 2 The colony morphology of tylosin (TYL)-degrading strain 2 (TYL2). The bacterial suspension of TYL2 was diluted to 10^{-7}. The microscopic morphology was observed under the 100 times of the oil immersion lens.

Table 1 Physiological and biochemical characteristics identification for tylosin (TYL)-degrading strain 2 (TYL2)

Item	Result	Item	Result
Catalase	+	2% NaCl	–
V-P reaction	–	5% NaCl	–
Citrate	+	pH=5.5	–
Hydrolyzed starch	–	pH=9.0	–
Glucose	–	15°C	–
Oxidase	–	50°C	+

+, positive; –, negative.

showed 99% similarity to *Brevibacillus borstelensis* strain UTM105 (KF952566.1) and 99% to *B. borstelensis* strain HBUM07014 (MF662440.1). Therefore, combination with the morphological characteristics of the TYL2, the bacteria strain TYL2 was identified as *Brevibacillus borstelensis*.

The growth curve of TYL2 was shown in Fig. 3. Generally, there were four stages contained in the growth curve, which were lag phase, logarithmic phase, stationary phase and decline phase, respectively. The first stage (0–9 h) with slow growth is lag phase. Rapid growth of *B. borstelensis* TYL2 was found within 9–27 h belonging to the logarithmic phase. It is consistent with majority strains (*Graclotolerans halotolerans* and *Rhodococcus ruber*) with the growth period of 5–30 h (Tang *et al.* 2008; Liu *et al.* 2015). The stationary phase was about 27–42 h. The decline phase was about 42–54 h.

3.2. Optimization of TYL degradation

Effect of temperature and oscillation frequency on the degradation of TYL Temperature is considered to be an important factor that affects the microbial growth of all eco-physiological parameters (Liu *et al.* 2016). The degradation rates of TYL at different temperatures were shown in Fig. 4-A. According to the results, compared with the control group, temperature had a significant influence on the degradation capacity of *B. borstelensis* TYL2. During the whole process of the temperature design, the degradation rate of TYL by TYL2 gradually increased then decreased with increases of the temperature. The maximum TYL degradation by TYL2 with the rate of 66.05% was observed at 35°C. The degradation rates of TYL by TYL2 decreased along with the increased temperature at a range of 40–45°C. The results suggest that temperature has a significant regulating role for the metabolism and enzyme of microorganisms (Wu *et al.* 2009). The enzymatic reaction rate increased by 1–2 times, and the activity of the microbe was increased when the temperature increased every 10°C until the most ideal temperature (35°C). After temperature exceeds this range, enzyme activity in the microbe decreased, and in addition, may have caused microbe protein degeneration (Takayama *et al.* 2006; Bird *et al.* 2008; Lan *et al.* 2011).

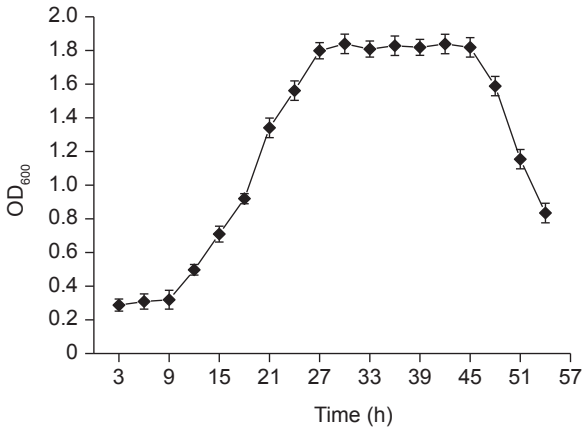

Fig. 3 Growth curve of tylosin (TYL)-degrading strain 2 (TYL2). Each point represents the concentration of *Brevibacillus borstelensis* TYL2 in cultural medium. The mean values and standard deviations (error bars) are presented (*n*=3).

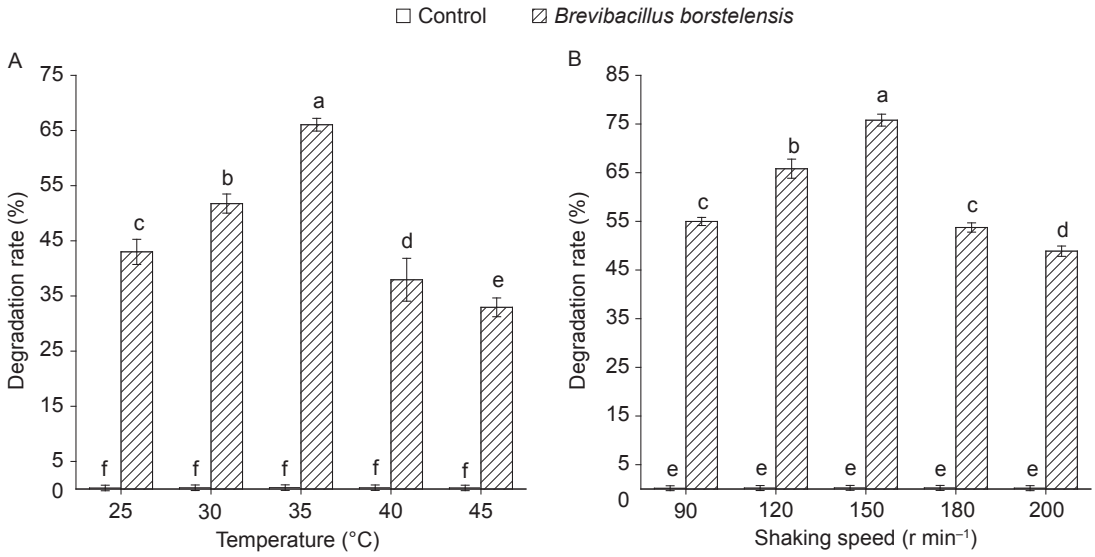

Fig. 4 Effects of temperature (A) and oscillation frequency (B) on the tylosin (TYL) degradation by TYL-degrading strain 2 (TYL2). The mean values and standard deviations (error bars) are presented (*n*=3). Different letters over bars indicate significant differences between different treatments, as determined by Duncan's test.

Also, the transport of substances could be accelerated at high temperature, however, the high temperature might also cause protein denaturation and enzyme deactivation secreted by bacteria, which may result in the decline of degradation rate of TYL by TYL2 strain. It is also noted that the degradation rates of TYL, at the temperature range 25–30°C, were lower than that at 35°C. This may be due to the fact that cellular metabolism changes rapidly with temperature changes and that a key enzyme was destroyed at relatively low temperatures, inhibited the growth rate of TYL2 (Minkevich *et al.* 2006).

The shaking speed was one of the major determinants of the growth of strain TYL2 in the medium. The degradation rates of TYL under the constant temperature were shown in Fig. 4-B. The effect of shaking speed on TYL was insignificant in the control group. With the increase in shaking speed, the degradation rates increased from 54.97% (90 r min^{-1}) to 75.80% (150 r min^{-1}) during this process. The degradation rates began to drop when the shaking speed exceeded 150 r min^{-1}, and the degradation rate declined to 48.87% at 200 r min^{-1}. Other studies have found similar results that the degradation rate increased with the acceleration of shaking speed and the biomass of microorganisms also increased (Liu *et al.* 2016). The reason for this situation might be that TYL2 is an aerobic microbe, which takes TYL as the only carbon source to promote growth and a large amount of oxygen is consumed. The aim of the shaking speed was to dissolve the molecular oxygen in the air into water to provide sufficient oxygen for microorganisms. The shaking speed could change the interaction between microorganism and culture medium (Lee *et al.* 1996). With the increase of shaking speed, the content of dissolved oxygen may be also increased; the growth rate of TYL2 was accelerated; and the degradation rate of TYL was enhanced. Once the oscillation frequency reaches 150 r min^{-1}, the dissolved oxygen also reaches a tipping point and the rate of TYL degradation reaches the maximum. The dissolved oxygen content could not increase and the degradation rate of TYL begins to decrease when the shaking speed continues to increase, which might because bacteria were sub-lethally or lethally injured or the structure of bacteria was changed. Therefore, the optimum shaking speed for *B. borstelensis* TYL2 to degrade TYL was 150 r min^{-1}.

Effect of initial TYL concentration and pH on the degradation of TYL The substrate of the medium (such as carbon sources and nitrogen sources) was one of the factors that influenced the growth rate of the strain. The concentration range in this paper refers to previous studies (Ma *et al.* 2015; Liu *et al.* 2016). The effect of the initial concentration of TYL was shown in Fig. 5-A. In the control group, the concentration of TYL changed insignificantly. The highest degradation rate of TYL was 75.14% at the initial concentration of 25 mg L^{-1}. The degradation rate decreased with the increase in initial concentration of TYL, which was similar to the results of previous study on algae (Uggetti *et al.* 2014). The reasons for this result might be that TYL with a concentration of 25 mg L^{-1} could provide sufficient nutrients for the growth of TYL2, but higher concentration of TYL was toxic for the microorganism and inhibited the growth of TYL2.

The pH was an important parameter in the process of antibiotics degradation, which not only affected the growth

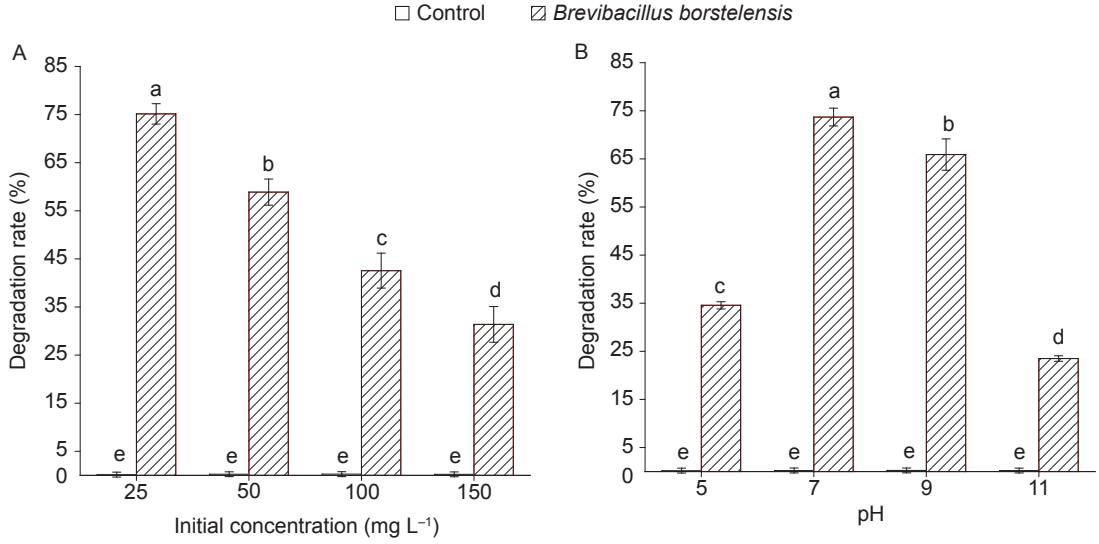

Fig. 5 Effects of initial concentration (A) and pH (B) on the tylosin (TYL) degradation TYL-degrading strain 2 (TYL2). The mean values and standard deviations (error bars) are presented (n=3). Different letters over bars indicate significant differences between different treatments, as determined by Duncan's test (P<0.05).

rate of bacteria, but also had an impact on the stability of antibiotics and bacterial strain. The effect of strain TYL2 on TYL degradation under the culture conditions of different pH values (5.0–11.0) was shown in Fig. 5-B. According to the results, the effect of pH on TYL degradation was insignificant in the control group, while degradation rate varied significantly between the treatments with different pH values. The optimal pH value for TYL degradation by TYL2 was 7.0 and the degradation rate was 73.70%. The degradation rates at pH values were in order of 7.0 (73.70%), 9.0 (56.90%), 5.0 (34.56%), and 11.0 (23.51%) for the degradation rates of TYL. The results indicated that the degradation of TYL was obviously impeded under acidic and alkaline conditions. This might be because that pH could change the charge properties on the surface of the microbial plasmalemma, and the permeability of the protoplasmic membrane to some ions, which had an effect on the ability of microorganisms to absorb nutriment and the metabolic activity. Therefore, the growth rate of TYL2 was inhibited in the condition of acid or alkaline. The secretases were reduced or hydrolyzed and the metabolites might also be altered, which reduced the degradation of TYL. In addition, under acidic or alkaline conditions, the structure of TYL was unstable and easily hydrolyzed and converted to another substance, resulting in reduced degradation rates. Each strain had its own pH range of adaptation to achieve the best degradation capacity (Yu *et al.* 2011). Pawar (2015) indicated that the degradation effect was the best when the soil pH was 7.5, and also showed that fungi were more tolerant to the acidic environment and bacteria were more adapted to the neutral. It was noteworthy that the strain TYL2 could be trialled as a candidate for soil bioremediation.

Effect of inoculum sizes on the degradation of TYL The degradation of TYL at different inoculum sizes of strain TYL2 was shown in Fig. 6. In the control group, no strain was added, so the degradation rate was not obvious within 7 days. Cultures inoculated with 2×10^8, 6×10^8, 1×10^9, 1.4×10^9, and 2×10^9 CFU removed 46.3, 62.27, 69.06, 74.18, and 65.43%, of TYL, respectively. It was obvious that the degradation rate of TYL reached the maximum at the inoculum size of 1.4×10^9 CFU. The degradation rate increased with the raise of inoculation size at the range of inoculum sizes from 2×10^8 to 1.4×10^9 CFU. The reason may be that the increasing number of bacteria shortens the logarithmic phase and speeds up the degradation of TYL (Yu *et al.* 2011). The large amount of inoculant *B. borstelensis* TYL2 could fastly adapt to the new environment by intraspecific and mutual assistance. However, TYL was used as the only carbon source, and there was a competitive relationship between microorganisms. Thus, the degradation of TYL was negatively affected on account of the decrease of biomass and metabolic capacity. The increasing inoculums of bacteria can shorten the degradation time of pollutants and reduce the growth of other bacteria. When the inoculation size was too large, the conditions such as oxygen and nutrients were limited, and the degradation efficiency of the pollutants would stop increasing (Xie *et al.* 2015).

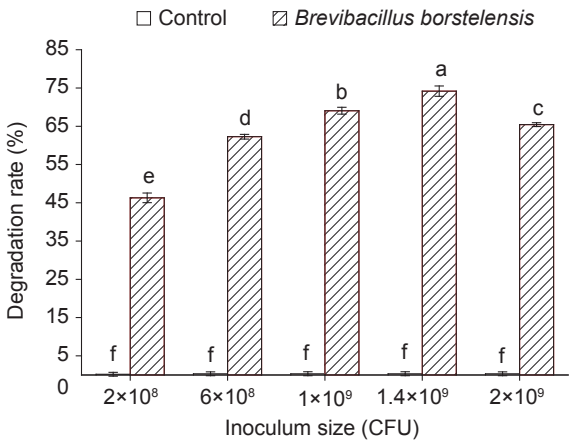

Fig. 6 Effects of inoculum sizes on the tylosin (TYL)-degrading strain 2 (TYL2). The mean values and standard deviations (error bars) are presented (n=3). CFU, colony-forming units. Different letters over bars indicate significant differences between different treatments, as determined by Duncan's test (P<0.05).

3.3. Degradation of TYL in sewage by TYL2

The degradation effects of TYL in sewage samples and mineral salts medium were shown in Fig. 7. In the control group, no strain was added, and the degradation rate was not obvious within 7 days. The degradation rates were enhanced over time in two environments. The degradation rates of TYL in sewage samples at different time were 34.05% (day 1), 46.00% (day 3), 54.36% (day 5) and 61.52% (day 7), respectively. And the degradation rates in mineral salts medium were 38.67, 53.93, 61.94 and 74.96%, respectively. Compared with the two environments, TYL in inorganic salts medium had better degradation rate than that in sewage. The same results have also been reported by Liu *et al.* (2017). One reason might be that the composition of sewage is more complex than inorganic salt medium, and TYL was no longer the only carbon source for the degrading strain. In addition, it was also possible that because some substances in sewage were toxic and inhibited the metabolism of the degrading bacteria, causing the consumption of TYL to slow down. At the same time, a mass of microorganisms in the sewage samples also compete with the degrading strain, which might reduce the content of degrading strain and eventually lead to low degradation rate.

Compared with microorganism degradation, abiotic

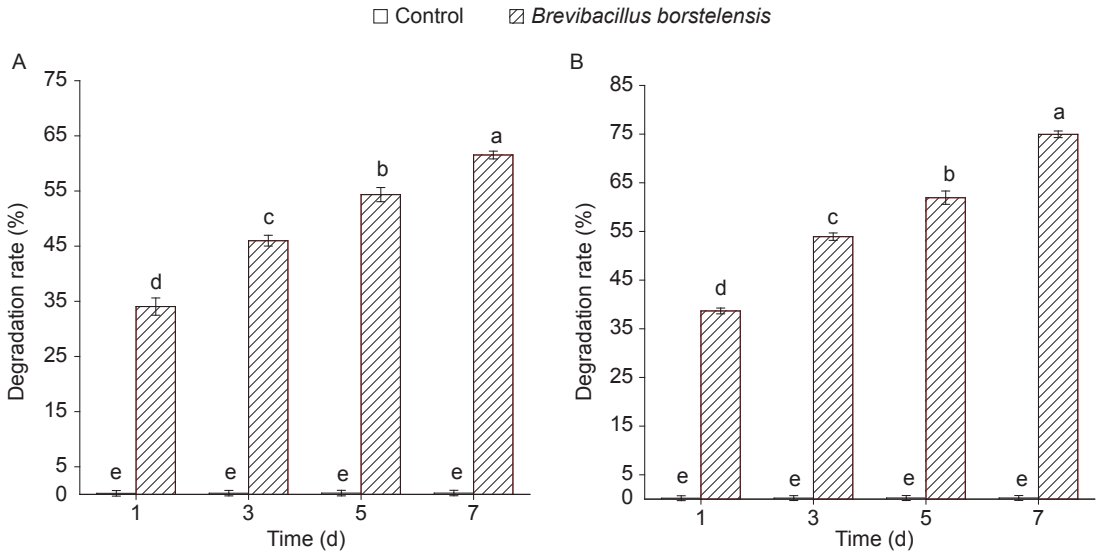

Fig. 7 Degradation rates of tylosin (TYL) by TYL-degrading strain 2 (TYL2) in sewage samples (A) and mineral salts medium (B) at 7 days. The mean values and standard deviations (error bars) are presented (n=3). Different letters over bars indicate significant differences between different treatments, as determined by Duncan's test (P<0.05).

removal methods, such as photodegradation and adsorption method etc., may show faster degradation of antibiotics (Sanscartier *et al.* 2011; Saeideh *et al.* 2014; Yin *et al.* 2018, 2019). However, microorganism degradation is based on the physiological conditions of themselves to decompose antibiotics, which can be operated without external force. As for photodegradation, pollutants usually absorb photons into an excited state under light conditions and then decompose (Reyes *et al.* 2006). However, they may also produce intermediate products, which may be more stable and more toxic than the antibiotics themselves in the photodegradation process (Vogna *et al.* 2004; Turiel *et al.* 2005; Carucci *et al.* 2006; Jiao *et al.* 2008). In the process of operation, the excessive use of ultraviolet light is also harmful to the human body. As for adsorption method, it could not remove antibiotics thoroughly, and sometimes inhibited the degradation of antibiotics in different environments (Li *et al.* 2017). Until now, many kinds of microorganism which could degrade particular antibiotics have been isolated (Migliore *et al.* 2012; Ma *et al.* 2015; Liu *et al.* 2016). In addition, different types of antibiotics may coexist in different environments. So it is also feasible to mix the different microorganism for antibiotic degradation. In particular, because of their small size and rapid reproduction, microbes can degrade antibiotics in many environments, including aquatic and soil environments (Schlusener and Bester 2006; Holly *et al.* 2008; Huang *et al.* 2012). And in consideration that microorganism degradation method is more economical, and could be easily operated and managed, it may be widely used in the antibiotics removal in the future.

4. Conclusion

A strain, named as TYL2, showing strong ability of degrading TYL, was isolated and identified as *B. borstelensis*. After the optimization of degrading conditions, more than 75% of TYL could be removed after 7 days under optimized conditions (35°C, 150 r min^{-1}, pH 7.0, 1.4×10^9 CFU of inoculum size, 25 mg L^{-1} of initial concentration). In addition, TYL2 could remove 61.52% of TYL in sewage environment. Overall, this research would provide theoretical basis and reference for the treatment process of the actual pollution containing TYL, and provide guidance for the degradation of other antibiotics. Whereas, the relative degradation mechanism and whether the degradation products will bring negative effects need to be further studied in the future.

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