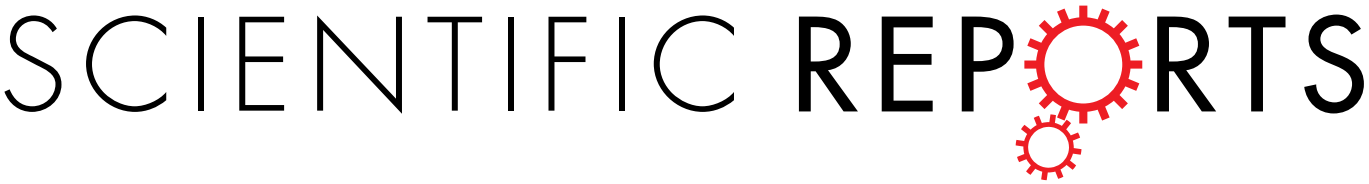

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Biodegradation of gentamicin by bacterial consortia AMQD4 in synthetic medium and raw gentamicin sewage

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Gentamicin, a broad spectrum antibiotic of the aminoglycoside class, is widely used for disease prevention of human beings as well as animals. Nowadays the environmental issue caused by the disposal of wastes containing gentamicin attracts increasing attention. In this study, a gentamicin degrading bacterial consortia named AMQD4, including *Providencia vermicola*, *Brevundimonas diminuta*, *Alcaligenes* sp. and *Acinetobacter*, was isolated from biosolids produced during gentamicin production for the removal of gentamicin in the environment. The component and structure of gentamicin have a great influence on its degradation and gentamicin C1a and gentamicin C2a were more prone to being degraded. AMQD4 could maintain relatively high gentamicin removal efficiency under a wide range of pH, especially in an alkaline condition. In addition, AMQD4 could remove 56.8% and 47.7% of gentamicin in unsterilized and sterilized sewage in a lab-scale experiment, respectively. And among the isolates in AMQD4, *Brevundimonas diminuta* BZC3 performed the highest gentamicin degradation about 50%. It was speculated that *aac3iia* was the gentamicin degradation gene and the main degradation product was 3′-acetylgentamicin. Our results suggest that AMQD4 and *Brevundimonas diminuta* BZC3 could be important candidates to the list of superior microbes for bioremediation of antibiotic pollution.

Gentamicin is a broad spectrum aminoglycoside antibiotic and China is a country with the largest gentamicin output in the world[1]. By the very nature of the case, a large amount of wastes containing gentamicin produced during gentamicin production and course of practical application in hospital and livestock farm were poured into the environment, which can induce development of gentamicin resistance genes[2–4]. The problem of drug resistance could reduce the function of antibiotics. Furthermore the large amount of gentamicin production solid waste and livestock excrements containing gentamicin have also been limited to be used as organic fertilizer. So far, few effective methods were exploited to deal with the gentamicin residues. Thus, it is critically urgent to develop economically feasible solutions for effectively removing or reducing the gentamicin residues in wastes and the environment.

The heat stable characteristic of gentamicin and its resistance to both acidic and alkaline conditions make it a big challenge to remove gentamicin from the environment using common chemical degradation or physical methods. Bioremediation, in contrast, is an attractive and successful cleaning technique for polluted environment[5]. And it is commonly considered that chemicals may be easily degraded by microflora via complementary transformation reactions[6]. Actually, microbial biodegradation and bioremediation technology has been increasingly applied to removal of antibiotics from the environment[7]. Selvi *et al*. isolated a kind of fungi named *Trametesversicolor* which could remove ciprofloxacin and norfloxacin more than 90%[8]. Islas-Espinoza *et al*. found that a consortium including *Bacillus licheniformis*, *Pseudomonas putida*, *Alcaligenes* sp. and *Aquamicrobium defluvium* could enhance the degradation of sulfonamides in soil[9]. However, the degradation of antibiotics depends on the various involved functional microflora[10]. So it is indispensable to isolate specific microorganism or consortia

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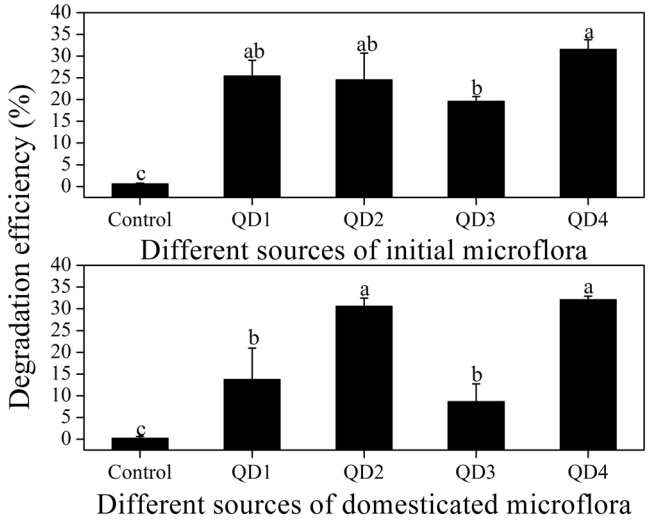

Figure 1. Degradation efficiencies of gentamicin by initial and domesticated microflora isolated from different sources of wastes (QD1, fermentation sludge; QD2, sludge maintained in anaerobic jar; QD3, sludge maintained in aerobic jar; bio-solids QD4). Condition: 1/5 BEP with 100 mg L^{-1} gentamicin incubated at 30 °C and 150 r/min orbital shaking. The mean values and SD (*error bars*) from triplicate trials are presented. *Data bars* having the same letter are not significantly different from each other at the 95% confidence level in the Duncan's test ($P < 0.05$).

for different antibiotics. To our knowledge, thus far, few of previous studies were conducted for the research about bioremediation of gentamicin or used for the treatment of raw gentamicin sewage.

In recent years, along with the development of sequencing technique, comparative genomics was more used in the study of functional genes, especially the virulence genes and resistance genes[11,12]. To provide information on gentamicin degradation genes and to evaluate its destruction mechanism, comparative genomics sequencing technique was used in this study. In this way more useful information including other kinds of antibiotics degradation genes and resistance genes could be further tapped.

In this case we supposed to develop an efficient microbial technology which will be applied for gentamicin removal from the environment. In consideration of the stability of consortia, this study was planning to domesticate and isolate effective gentamicin-degrading microflora. After the optimization of fermentation conditions, the removal ability of the microflora in raw gentamicin sewage was determined for the large-scale usage. In addition, the degradation ability of the isolated bacteria from the microflora was detected and the degradation genes and gentamicin destruction mechanism were identified and evaluated using comparative genomics sequencing technique.

Results

isolating of gentamicin-degrading bacterial consortia. As shown in Fig. 1, both degradation efficiencies of gentamicin in the 1/5 of beef extract peptone medium (BEP) inoculated with initial and domesticated microflora were significantly higher than those in controls ($P < 0.05$). The initial bacteria consortia before domestication from fermentation waste water (QD1), anaerobic jar sludge (QD2), aerobic tank sludge (QD3) and bio-solids sludge (QD4) could degrade gentamicin by 25.5%, 24.5%, 19.6% and 31.6% of initial gentamicin content, respectively. The degradation efficiency of acclimatized ones from QD1 and QD3 decreased to 13.7% and 8.6%, respectively. Hence these two types of microflora (QD1 and QD3) were not chosen in the following experiments due to low gentamicin degradation ability. In contrast, the degradation efficiency of mixed bacteria from QD2 and QD4 increased to 30.5% and 32.2%, respectively. Because of the higher degradation efficiency, acclimatized microflora in QD4 (AMQD4) was considered for further degradation studies. In addition, no significant changes were found for optical density of medium at 600 nm (OD600) and gentamicin contents in MSM (data not shown) over the domestication period.

Optimization of gentamicin degradation by AMQD4. The medium concentrations had significant ($P < 0.05$) effects on the gentamicin degradation by AMQD4 (Fig. 2a). The maximum gentamicin degradation efficiency with a value of about 38.6% was obtained in 1/5 of BEP medium. The degradation efficiency of gentamicin by AMQD4 decreased when the medium was diluted from 1/5 to 1/20 of BEP (Fig. 2a). In addition, pH values and bacterial growth (OD600) at the end of fermentation decreased significantly ($P < 0.05$) from 9.1 to 7.9 (Fig. S1a) and from 4.0 to 0.12 (Fig. S2a), respectively, when the medium was diluted from 1/1 to 1/20 of BEP.

At the optimized medium concentration of 1/5 of BEP, the degradation efficiency of gentamicin by AMQD4 decreased with concentration of gentamicin increasing in the medium (Fig. 2b). For example, the gentamicin degradation efficiencies were significantly ($P < 0.05$) increased from 7.0% to 40.8% when the gentamicin concentrations in the medium were reduced from 400 mg L^{-1} to 50 mg L^{-1}. It was found that AMQD4 could grow well and reach the largest biomass (OD600 = 0.64) in the medium containing 100 mg L^{-1} of gentamicin and could keep active growth (OD600 = 0.45) even in medium with high dose of gentamicin (400 mg L^{-1}) (Fig. S2b). It is