academicJournals

Vol. 13(22), pp. 2227-2230, 28 May, 2014
DOI: 10.5897/AJB2013.12836
Article Number:
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Biodegradation of norfloxacin by *Penicillium frequentans* isolated from polluted soil

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Received 24 May, 2013; Accepted 16 May, 2014

One norfloxacin-degrading fungi was isolated from soil contaminated by norfloxacin and preliminary identified as *Penicillium frequentans*. Indoor simulative degradation experiments were carried out to investigate the biodegradation kinetics of norfloxacin with or without NFX3 in soil. The results indicate that the biodegradation of norfloxacin were both in accordance with first-order kinetics equation whether the NFX3 existed or not, and the microbial degradation rate constant of norfloxacin with NFX3 in soil was 0.0121 day^{-1}.

Key words: Norfloxacin, soil, biodegradation, microbial consortium.

INTRODUCTION

Quinolones are known as a group of synthetic organic antibiotics, and extensively used in agriculture to prevent diseases in livestock and treat illness; therefore, soil and groundwater body have been badly contaminated (Kay et al., 2005). Some measures revealed that the dominant veterinary drugs used in China are antimicrobial drugs, especially sulfonamides, macrolides and quinolones (Yu et al., 2012). Nowadays, systematic studies on the accumulation, transportation, and transformation of veterinary drugs in aquatic and terrestrial environment, as well their effects on various organisms are still scarce.

Consequently, it is critical to investigate the environmental behavior of veterinary drugs, which would be helpful for accessing the security of veterinary drugs utilized in aquatic and terrestrial environment, and modifying the contaminated soil. In predicting the transport of quinolones in the environment, and assessing their risk to terrestrial and aquatic ecosystems, it is necessary to know the biodegradation data of quinolones, but only a limited amount of biodegradation data of quinolones have been reported in the literature (Baran et al., 2006; Peng et al., 2006). In this study, biodegradation for norfloxacin in soil was carried out to investigate the biodegradation kinetics.

MATERIALS AND METHODS

Norfloxacin (NFLX), obtained from Daming Biotechnology Co. Ltd., China was further purified by recrystallization from aqueous solutions. After filtration and drying, its purity was determined by UV spectrometry (type UV-2401PC, Shimadzu Co. Ltd, China), to be 0.996 in mass fraction. H_2SO_4 and NaOH used in experiments were all analytical reagents.

The soil sample was collected from Xingyang in China. Its physical and chemical properties show that the pH value is 7.82, which indicate that it is alkaline and the organic matter content is not small (11.0 g/kg).

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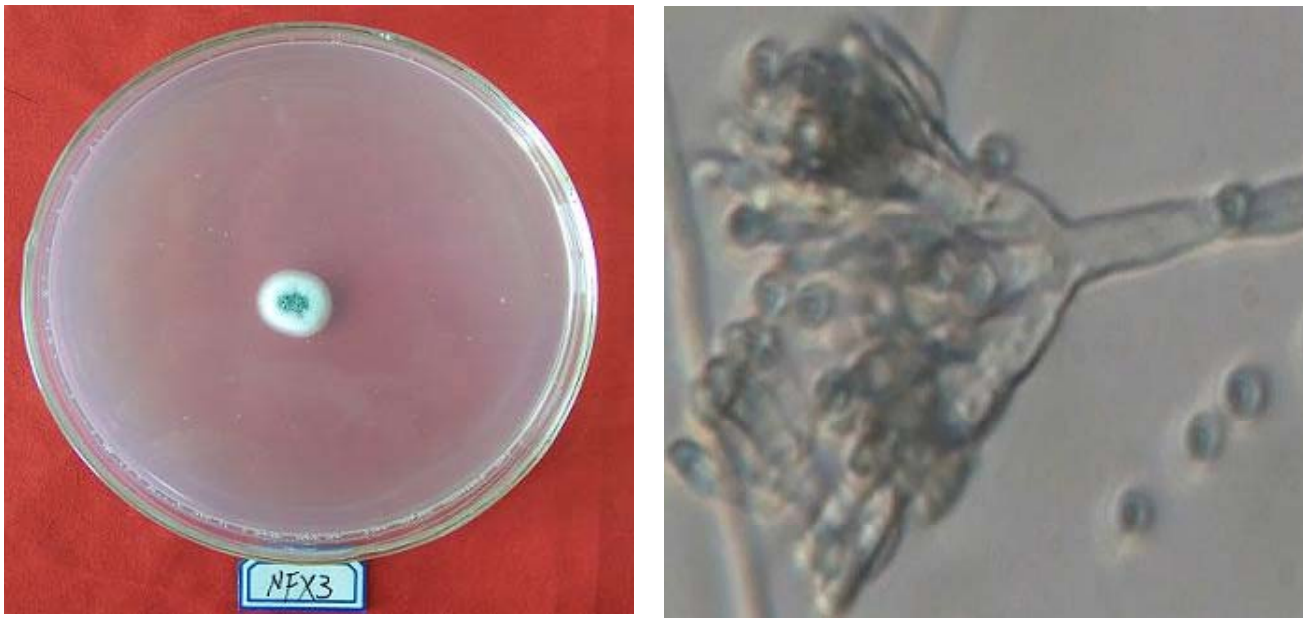

Figure 1. Colony shape feature and microscopic observation (10×40 times) on bacteria isolated.

Isolation and identification of bacteria

Ten grams of the norfloxacin contaminated soil sample was added to 90 ml sterile distilled water, and the suspension was shaken vigorously at 303.15 K and 120 r/min for 30 min. Serial dilutions (10^{-2}-10^{-4}) were prepared using sterilized distilled water, and 0.1 ml aliquots were inoculated in Petri dishes that contained potato dextrose agar medium (glucose, 20.0 g; potato extract, 200.0 g; agar, 20.0 g; distilled water, 1000 ml), beef extract peptone agar medium (beef extract, 5.0 g; peptone, 10.0 g; NaCl, 5.0 g; agar, 20.0 g; distilled water, 1000 ml; pH 7.2-7.4) and Gause I agar medium (soluble starch, 20.0 g; KNO_3, 1.0 g; NaCl, 0.5 g; $K_2HPO_4 \cdot 3H_2O$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; agar, 20.0 g; distilled water, 1000 ml). The pH of the media was adjusted to 7.0-7.2 with 0.1 mol/L HCl prior to sterilization (YXQ-LS-30S vertical pressure steam sterilizer, Shanghai Boxun Medical Equipments Co. Ltd., China) at 394.15 K for 20 min. Each medium was supplemented with 10.0 mg/L of norfloxacin to inhibit the growth of Gram-negative bacteria and fungi, respectively.

Following inoculation, the plates were incubated at 303.15 K for up to seven days, and colonies were purified without antibiotics by streaking onto the respective agar medium from which the colonies were isolated, and selected isolates of bacteria were preserved on nutrient agar medium at 277.15K for further experiments. Microbial selection was based on colony morphology, color and presence of diffusible pigments according to Bergey's manual of systematic bacteriology (Malghani et al., 2009).

The selected strain was transferred to mineral salt medium (MSM) (Herman and Frankenberger, 1999) containing 10.0 mg/L norfloxacin in a 250 ml Erlenmeyer flask and incubated on an orbital shaker (Shanghai Yuejin Medical Apparatus Factory) at 303.15 K and 120 r/min for 3-5 days. Then, growth was observed and the culture (1 ml) was shifted to fresh MSM containing 20.0 mg/L norfloxacin. In the same way, the culture was transferred to serially increasing concentrations of norfloxacin up to 30.0 mg/L.

Degradation experiments

Nearly 4.0 g of each soil sample was added to 250 ml conical flask with plug. Following, norfloxacin solution was added up to 50 mg/kg; 1 ml NFX3 bacterial suspension was added or not; appropriate amount of water was reentered up to 60% of maximum water holding capacities in field. Then lucifuge degradation experiments enclosed in constant temperature incubator (Shanghai Yuejin Medical Apparatus Factory) at 298.15K was processed. Thereafter, each soil was sampled at intervals (0, 5, 10, 20, 30, 40 and 60 d), with 100 ml 0.1 mol/L NaOH extracting solution being affiliated to oscillate (140 r/min) and extract at 298.15 K for 24 h. After centrifugal separating (4000 r/min; type LD4-2A, Beijing Medical Appliance Factory), supernatant liquor was taken to determine the norfloxacin concentration using UV spectrophotometry (Diaz-Cruz et al., 2003), and residual quantity of norfloxacin in soil was calculated.

RESULTS AND DISCUSSION

Isolation and identification of bacteria

Five different microorganisms were isolated from the norfloxacin-exposed soil samples by the above mentioned method. All isolates were fungi and had high tolerance to norfloxacin. Among them, strain NFX3 was preliminary identified as *Penicillium frequentans*, and further confirmation will be made by sequencing of their 16 SrRNA gene. The colony shape feature and microscopic observation are shown in Figure 1.

Degradation of norfloxacin with or without NFX3 in soil

Based on the degradation curves of norfloxacin and their correlation coefficients (r = 0.95-0.99), its degradation process could be described by the first order kinetic

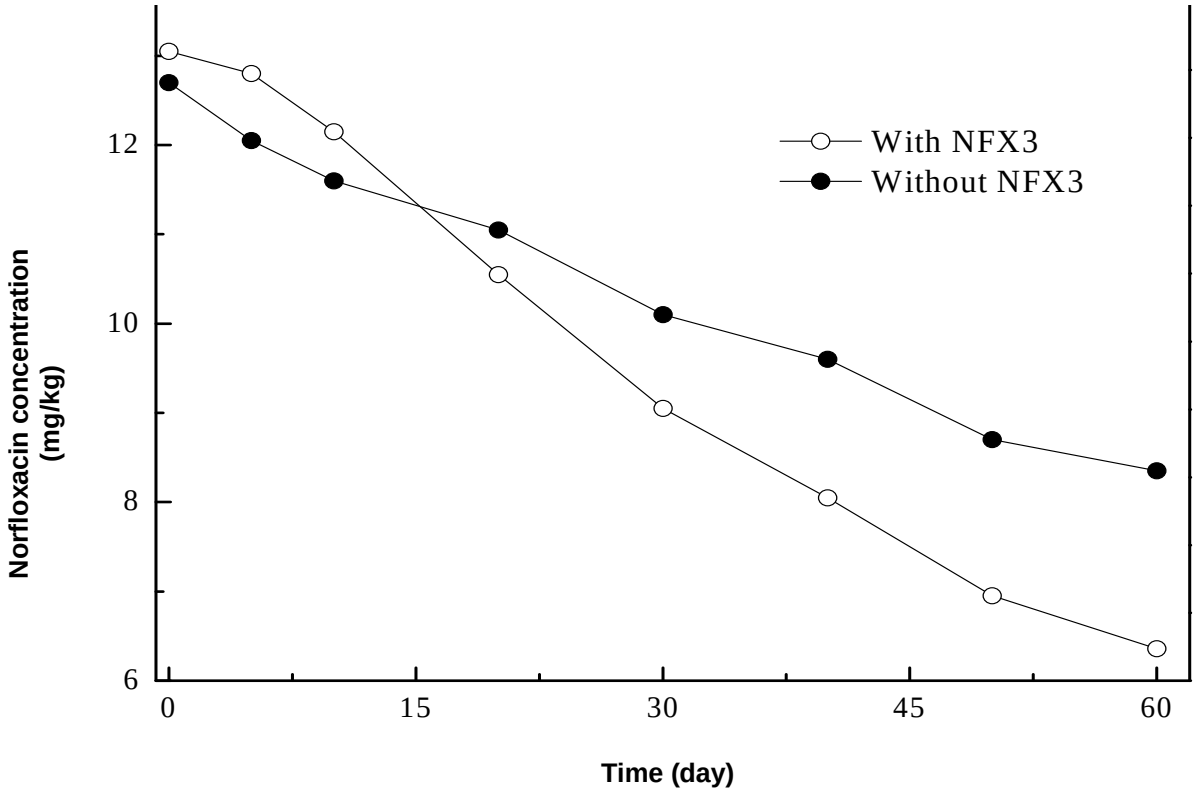

Figure 2. Degradation dynamics of norfloxacin with or without NFX3 in soil. *c* = norfloxacin concentration, *t* = time (day).

Table 1. Kinetic parameters of norfloxacin degradation with or without NFX3 in soil at 298.15 K.

Treatment	$\ln(c_0/c)=kt$	*r*	*k* (day^{-1})	$t_{½}$ (day)
Without NFX3	$\ln(c_0/c) = 0.0072t$	0.9943	0.0072	96.3
With NFX3	$\ln(c_0/c) = 0.0121t$	0.9935	0.0121	57.3

c = norfloxacin concentration; c_0 = initial norfloxacin concentration; *k* = degradation rate constant; *t* = time (day), $t_{1/2}$ half-life (day).

equation (Zhang et al., 2004).

$$\ln \frac{c_0}{c} = kt \quad (1)$$

$$t_{1/2} = \frac{\ln 2}{k} \quad (2)$$

Where, *c* is the norfloxacin concentration at *t*, mg/kg, c_0 is the initial norfloxacin concentration, mg/kg, *k* is the degradation rate constant, day^{-1}, *t* is time, day, $t_{1/2}$ is the half-life, day.

The degradation dynamics of norfloxacin with or without NFX3 in soil at 298.15K is shown in Figure 2 and Table 1. It is shown in Table 1 that the biodegradation of norfloxacin with or without NFX3 were both in accordance with first-order kinetics equation, and the microbial degradation rate constant of norfloxacin with NFX3 in soil was 0.0121 day^{-1}, which is probably dependent on stability and bacterial inhibition for norfloxacin in soil (Bel et al., 2009). From Figure 2, the degradation efficiency of norfloxacin with NFX3 was slightly lower than without NFX3 in the first 15 day, which may be that the NFX3 still could not meet the new living environment; but 15 days later, the former was significantly higher than the latter, NFX3 could have been domesticated and adapted to the soil environment. Faster degradation of norfloxacin occurred after the exponential growth phase, probably due to acetate consumption since the presence of such easily degradable carbon source allowed the increase in the biomass of the degrading strain, accelerating the biodegradation process. The degradation efficiency of norfloxacin with or without NFX3 in soil at 60 day reached 51.3 and 34.2%, respectively, while the initial norfloxacin

concentration was 13.0 g/kg. The results showed that NFX3 promoted significantly norfloxacin degradation.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by the Henan Province Natural Science Foundation of China (Project No. 0611033400).

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