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Influence of erythromycin A on the microbial populations in aquaculture sediment microcosms

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Abstract

Degradation of erythromycin A was studied using two sediment samples obtained from the salmon and trout hatchery sites at Hupp Springs (HS) and Goldendale (GD), Washington, United States. The former site had been treated for 3 years with erythromycin-medicated feed prior to the experiments, and the latter site had not been treated with any antibiotic for at least 6 years. The two sediment microcosms treated with either *N*-[methyl-^{14}C]erythromycin A or [1,3,5,7,9,11,13-^{14}C]erythromycin A showed *S*-curves for erythromycin A mineralization with a prolonged lag time of 120 days, except for GD microcosms treated with [1,3,5,7,9,11,13-^{14}C]erythromycin A. We proposed a simplified logistic model to interpret the mineralization curves under the assumption of the low densities of initial populations metabolizing erythromycin A. The model was helpful for knowing the biological potential for erythromycin A degradation in sediments. Although erythromycin A added to the two sediment microcosms did not significantly alter the numbers of total viable aerobic bacteria or erythromycin-resistant bacteria, it affected the bacterial composition. The influence on the bacterial composition appeared to be greater in GD microcosms without pre-exposure to antibiotics. PCR-RFLP and DNA sequence analyses of the 16S ribosomal RNA gene and the erythromycin esterase (*ere*) gene revealed that *ereA* type 2 (*ereA*2) was present in potentially erythromycin-degrading *Pseudomonas* spp. strains GD100, GD200, HS100, HS200 and HS300, isolated from erythromycin-treated and non-treated GD and HS microcosms. Erythromycin A appeared to influence the development and proliferation of strain GD200, possibly via the lateral gene transfer of *ereA*2.

Keywords: Erythromycin A; Degradation; Erythromycin esterase; Microcosm; *Pseudomonas*; Restriction fragment length polymorphism

1. Introduction

The use of antimicrobial agents in food-producing animals is a public health concern because of the potential adverse effects of antimicrobial residues on the development of antibiotic-resistant bacteria and the transfer of resistance genes to human pathogens (Alderman and Michel, 1992; Pothuluri et al., 1998). Of 21 vet-

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erinary and human antibiotics, erythromycin is the most frequently detected in 139 US stream sites that are considered susceptible to contamination from human and industrial wastewater (Kolpin et al., 2002). The great frequency of detection of erythromycin may be due to significant use in food-producing animals. A large amount of poorly adsorbed or metabolized erythromycin is released into the aquatic environment. Eguchi et al. (2004) reported that erythromycin strongly inhibits microalgae, e.g., *Selenastrum capricornutum* (EC_{50} = 0.037 mg/l).

Discharged erythromycin is partly converted to the anhydride form by acid- and base-catalyzed reactions in aqueous solutions (Atkins et al., 1986; Cachet et al., 1989; Kim et al., 2004a). The protonated compounds of erythromycin and its degradation products are likely adsorbed on soil components, mainly by cation exchange and hydrophobic interactions (Kruger, 1961; Kim et al., 2004b). The adsorption processes lead to the persistence of erythromycin compounds in sediments. In addition, some bacteria are able to degrade erythromycin A and related compounds by erythromycin esterase activity (Kim et al., 2002, 2004c). Such behavior of erythromycin in aquatic environments results in significant decrease of biological activity against microorganisms. Therefore, evaluation of the potential impact of erythromycin on the ecosystem must consider the chemical and biological factors affecting degradation.

However, there is little information about the impacts on microbial populations. The aim of this study was to investigate the impact of erythromycin A on microbial populations during degradation in sediment microcosms monitored with two differently ^{14}C-labeled erythromycins A (Fig. 1). To better understand the behavior of microbial populations degrading erythromycin A, we proposed a logistic equation with a generalized mineralization rate constant, a delayed time to the mid-point of the curve, and the initial mineralization that occurs during the lag time. The populations of aerobically growing bacteria with densities of $>10^4$ colony-forming units per gram-soil were investigated numerically based on the phenotypes and antibiotic susceptibilities. Among them, five potentially erythromycin-degrading strains were subjected to restriction fragment length polymorphism (RFLP) and DNA sequence analyses of the PCR-amplified 16S ribosomal RNA gene and the erythromycin esterase gene.

2. Materials and methods

2.1. Chemicals

Erythromycin A was purchased from the Aldrich Chemical Co., Milwaukee, WI. To examine the microbial activities for the degradation of the *N*-methyl group and the macrocyclic lactone ring of erythromycin A, two differently ^{14}C-labeled erythromycins A were used: *N*-[methyl-^{14}C]erythromycin A (Fig. 1A, specific activity, 54 mCi mmol^{-1}) with a purity of 98% was

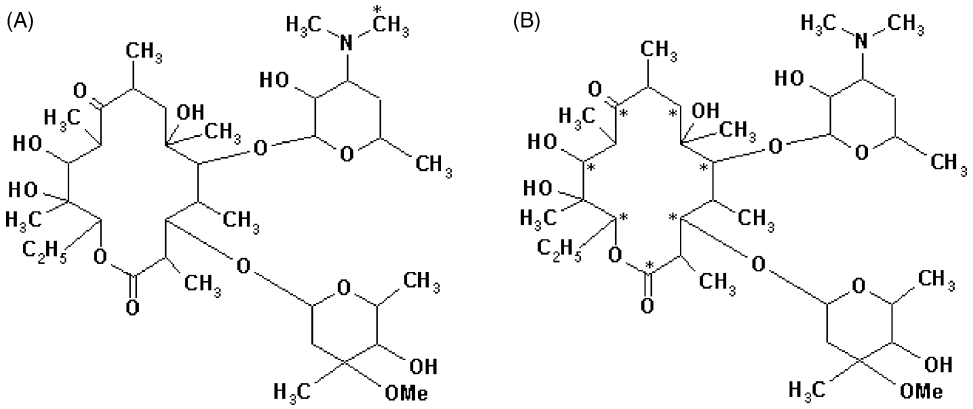

Fig. 1. Structures of *N*-[methyl-^{14}C]erythromycin A (A) and [1,3,5,7,9,11,13-^{14}C]erythromycin A (B). The ^{14}C-labeled carbon positions are shown by asterisks.