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SORPTION OF TYLOSIN A, D, AND A-ALDOL AND DEGRADATION OF TYLOSIN A IN SOILS

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Abstract—Heightened concerns regarding the potential impact on soil and water quality of veterinary antibiotics warrant a better understanding of the environmental fate of antibiotics in soil. Sorption of the macrolides tylosin A (TA), tylosin D, and TA-aldol was measured in several soils and evaluated with respect to soil pH, organic matter content, percentage clay, and cation-exchange capacity (CEC). Tylosin and related compounds exhibit similar sorption characteristics and generally are strongly sorbed, with sorption being well and positively correlated to surface area, clay content, and CEC. Sorption coefficients normalized by CEC were within a narrow range ($10^{4.1\pm0.21}$ L/mol$_c$) for all but one soil; however, good extraction recoveries with only methanol for most soils suggested that hydrophobic processes also contribute to sorption. Aerobic degradation of TA over a three-month period in two freshly collected agricultural soils and ^{60}Co-irradiated soils indicated that both abiotic and microbial processes contribute to TA transformation. The abiotic process was much slower and dominated in the first two weeks, followed by rapid microbial degradation within 3 d. Three primary degradation products were identified using liquid chromatography with full-scan mass spectrometry, with unconfirmed identifications of TA having the aldehyde group oxidized to an acid (m/z = 932) in both soils and tyslosin B (m/z = 772) as well as tylosin B having the aldehyde group oxidized to an acid (m/z = 788) in the sandy soil.

Keywords—Veterinary antibiotics Cation-exchange normalized sorption Half-life

INTRODUCTION

At swine production facilities, animals are given antibiotics for growth promotion and health maintenance. These antibiotics and their metabolites are excreted in waste products that ultimately are applied to arable land as fertilizer. Antibiotic concentrations in animal waste products typically are below the levels that could cause acute environmental impact [1]. However, increases in the use of antibiotics in concentrated animal feeding operations and land application of manure are increasing our awareness regarding the potential for development and dissemination of antibiotic-resistant pathogens [2]. Additional concerns include the possibility that changes in microbial populations caused by antibiotic exposure might undermine the ability of microbes to degrade contaminants such as pesticides [3] or have deleterious effects on important chemical cycles such as nitrification [4,5]. These issues illustrate the importance of assessing the environmental fate of antibiotics used in concentrated animal feeding operations.

Tylosin, a wide-spectrum, macrolide antibiotic produced by fermentation of *Streptomyces* strains [6], is a veterinary antibiotic (Tylan®; Elanco Animal Health, Greenfield, IN, USA) widely used in the United States at therapeutic and subtherapeutic levels and/or for increases in rates of weight gain and improved feed efficiency in companion animals, cattle, chickens, turkeys, cattle, and swine [7]. Tylosin is one of the most common antibiotics present in swine and turkey manures, with concentrations as high as 4 mg/L measured in swine manure [8]. Tylan is a mixture containing 80 to 90% tylosin A (TA) and lesser amounts of tylosin B (TB), tylosin C (TC), and tylosin D (TD), all of which contribute to its potency [9]. Other minor constituents include lactenocin [10], 5-O-mycaminoseyltylonolide [11], and desmycinosyl tylosin [12]. The molecular structure (Fig. 1) of the various components of tylosin are characterized by a substituted, 16-membered lactone ring with an amino sugar (mycaminose) attached to the lactone ring at position 5 via a β-glycosidic linkage. Tylosin A, TC, and TD all contain mycinose, attached at position 14 of the ring, and mycarose, attached at position 4 of the mycaminose moiety. The remaining related substances contain either one or neither of these two sugars. Tylosin A and related compounds are stable at pH 4 to 9 [13]. Below pH 4, the sugar moieties are cleaved from the parent molecule, forming compounds of lower molecular weight. Above pH 9, loss of the sugars can occur as well as condensation at the R_1 aldehyde group of TA, TB, and TC, forming the corresponding aldols [14].

Tylosin is metabolized after administration to livestock (via injection or through feed), with TB, TD, and dihydrodesmycosin metabolic residues typically found in feces along with the main active ingredient, TA [15]. When Tylan is administered as a feed additive, much less TA is metabolized because of the relatively low oral bioavailability of TA [16] (http://www.emea.eu.int/). Tylosin A metabolites retain bioactivity, with antimicrobial potencies of 83, 35, and 31% relative to TA for TB, TD, and dihydrodesmycosin, respectively [17]. The current practice at swine production facilities is to store wastes in an open lagoon (conventional) or a covered lagoon. Lagoons typically are dewatered one or more times per year ([18]; http://www.aphis.usda.gov). The liquid manure slurry obtained is then applied to arable land as fertilizer [9]. Half-lives of less than 8 d have been reported for TA and its metabolites in manure slurries, with longer half lives noted for anaerobic manure systems [9,15,17,19]. The disappearance of tylosin from manure appears to result not only from microbial and abiotic degradation but also from increasingly stronger or

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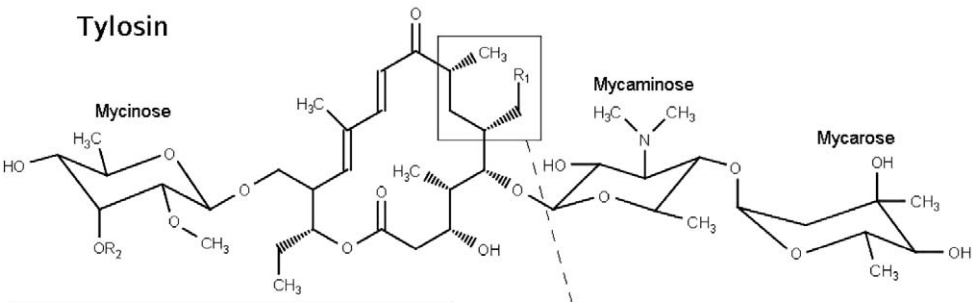

	R1	R2	Mycarose	Mycinose
Tylosin A	CHO	CH_3	+	+
Tylosin B	CHO	CH_3	-	+
Tylosin C	CHO	H	+	+
Tylosin D	CH_2O	CH_3	+	+
OMT	CHO	NA	-	-
DMT	CHO	NA	+	-

+ = sugar present
- = sugar not present

Fig. 1. Molecular structure of tylosin and tylosin degradation products.

irreversible binding of tylosin and metabolites onto manure solids over time [9,15]. Extraction of manure amended with [^{14}C]tylosin before and after a 30-d incubation showed an increase in nonextractable ^{14}C residues from 19 to 53% for cattle excreta and from 22 to 36% for swine excreta [17]. Tylosin half-lives measured in soils ranged from 6.1 d to three months [20–22], and from 17 to 54 d in surface-water samples with a variable lag time of 0 to 60 d [23].

Tylosin, as a weak base with a pK_a of 7.7 [24], is expected to sorb primarily through cation-exchange interaction; however, because of its large size, some partitioning through hydrophobic forces also may be expected (estimated octanol–water partition coefficient, 43L/L) [24]. Other potentially important sorption mechanisms occurring at the clay surface may include hydrogen bonding of tylosin carboxyl groups with cation-associated water molecules and hydrophobic interaction with the siloxane surface [25]. To date, few studies have been performed to evaluate the sorption characteristics of tylosin [8,25–28]. Rabolle and Spliid [26] as well as Kumar et al. [8] measured sorption of tylosin on agricultural soils from Denmark and Minnesota (USA), respectively. Sorption was found to be somewhat nonlinear and correlated to clay content, with soil–water distribution coefficients (K_d) in the order of 10^1 to $10^{2.1}$ L/kg. For swine manure, sorption magnitudes were similar, with K_d values ranging from $10^{1.6}$ to 10^2 L/kg and the manure particles from the conventional open lagoon exhibiting the highest sorption [27,28].

During a field study in which tylosin was applied to a field both with and without manure, tylosin half-life was reduced from 7.1 to 4.5 d in the presence of manure, and no tylosin was ever detected below a depth of 25 cm [22]. Tylosin A, however, has been found in surface waters, albeit at concentrations less than 1 μg/L [3,29–31], which is orders of magnitude lower than any inhibitory concentration reported for various biota [8]. Nevertheless, much uncertainty remains concerning the fate of the degradation products of TA, which retain significant antimicrobial activity. To our knowledge, no studies have included the environmental concentrations or the environmental fate of TA breakdown products. These bioactive metabolites are less hydrophobic and, hence, potentially more mobile in environmental systems.

The purpose of the present study was to obtain a better understanding of the environmental fate of tylosin in agricultural soils. Sorption of TA and two of its degradates, TD and TA-aldol, was measured in several soils and evaluated with respect to soil pH, organic carbon (OC) content, clay content, and cation-exchange capacity (CEC). Aerobic degradation of TA in moist soil microcosms over a three-month incubation period was measured in a low-OC sandy soil and a higher-OC silty clay loam, and compared to degradation in ^{60}Co-irradiated soils to determine the relative contributions of biotic and abiotic processes.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical reagent grade or higher purity. Tylosin tartrate and sodium perchlorate were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Tylosin D and TA-aldol were synthesized as described in the next section. Ammonium acetate, calcium chloride dihydrate, sodium hydroxide, sodium phosphate dibasic, tetrabutylammonium hydrogen sulfate, acetonitrile, and methanol were purchased from Mallinckrodt Baker (Paris, KY, USA). Phosphoric acid (85%) was purchased from EM Science (Gibbstown, NJ, USA). Water was purified using reverse osmosis and a Nanopure system (resistance, ≥18 MΩ; Barnstead International, Dubuque, IA, USA). All standards and solutions were protected from exposure to light and used within 24 h of preparation or stored at approximately 4°C for no more than 3 d.

Preparation of tylosin degradates

Tylosin D was synthesized from tylosin tartrate by reduction with sodium borohydride in ethyl acetate. Tylosin tartrate (120 mg, 113 μmol) and sodium borohydride powder (9 mg, 238 μmol) were added to ethyl acetate (35 ml) in a 100-ml Erlenmeyer flask. The mixture was stirred, and the solution was periodically assayed by high-performance liquid chromatography (HPLC) to determine the progress of the reaction. After 5 h, all the TA had been reduced to a single product. The reaction mixture was washed with water (50 ml), and the water was extracted three times with ethyl acetate. The ethyl acetate fractions were combined, dried over anhydrous magnesium sulfate, and evaporated to dryness. The residue was then dissolved in toluene (<5 ml), and hexane was added (<25 ml) until a white precipitate formed. The mixture was centri-

Table 1. Physical and chemical properties of selected soils

Soils	Isotherm soil to water ratio (g/ml)	pH[a]	% OC[b]	% Clay	Surface area[c] (m^2/g)	CEC[d] (cmol/kg)	Clay type[e] (g/kg)			
							S	V	I	K
Eustis-25[fg]	4:35	5.5 (5.8)	0.46	<3	1.0	0.76[h]	—	—	—	Primary
Bloomfield-3[ij]	2:35	6.4 (6.1)	0.36	8	13.5	4.4[g]	32	12	15	5.4
Chalmers-6[ij]	2:35	6.5 (6.7)	1.17	16	57.6	13.0[g]	96	33	33	14
Milford-21[ij]	2:35	7.4 (7.5)	0.70	36	56.9	11.2		Mixed clay types		
Drummer-1[ij]	2:35	7.2 (7.2)	2.91	21	85.4	26.5[g]		Mixed clay types		
EPA-23[k]	0.5:35	6.7 (6.4)	2.38	69	177	31.1[l]	576	—	42	73
Toronto-4[j]	2:35	4.4 (4.4)	1.34	21	75.4	11.2[g]		Mixed clay types		
Drummer-30[m]	NA[n]	7.9	2.2	33	NA	16.4		Mixed clay types		
Oakville-31[m]	NA	6.9	0.87	11	NA	4.4	—	—	—	—

[a] 1:1 (g/ml) soil:water pH followed by measured isotherm pH values in parentheses.
[b] OC = organic carbon.
[c] Surface areas determined by the U.S. Salinity Laboratory (Riverside, CA) using ethylene glycol monoethyl ether adsorption as described by Cihacek and Bremner [36].
[d] CEC = cation-exchange capacity.
[e] S = smectite; V = vermiculite; I = illite; K = kaolinite.
[f] Clay mineralogy data from http://ssldata.nrcs.usda.gov/querypage.asp.
[g] CEC at the native soil pH.
[h] Estimated from data for a similar soil in which pH-dependent CEC were determined [37].
[i] Clay mineralogy data from Cox [38].
[j] Soil characterization except for clay mineralogy from Li [39].
[k] Soil characterization data from [40].
[l] CEC by pH 7 ammonium acetate extraction.
[m] Soil characterization data from A & L Great Lakes Labs (Forte Wayne, IN, USA).
[n] NA = not applicable.

fuged at 630 *g* for 20 min, and the supernatant was discarded. The remaining white crystals were dried for 72 h in a vacuum desiccator. The product assayed at 93% purity by HPLC and had a mass spectrum consistent with that of TD.

Tylosin A-aldol was synthesized from tylosin tartrate using a modification of a previously published method [14]. Tylosin tartrate (0.6 g) was dissolved in 5 ml of 50:50 (v:v) water/ethanol and pH adjusted to 10 with aqueous sodium hydroxide. The reaction mixture was placed in a warm water bath (55°C) for 20 d, and aqueous NaOH was added periodically to maintain pH 10. Tylosin A-aldol was isolated from the reaction mixture by semipreparative HPLC using a reverse-phase column (length, 250 mm; inner diameter, 10 mm; 5 μM, particle size; Dynamax Microsorb C_8; Rainin Instrument, Woburn, MA, USA), with a mobile phase containing 60:40 2.25% sodium perchlorate (pH 2.25)/acetonitrile at a flow rate of 3 ml/min. The fraction containing TA-aldol was collected in a vial and the pH adjusted to 10 with phosphate buffer and NaOH. Solid-phase extraction was performed to remove the mobile-phase salts using a SDB-L cartridge (3 ml, 500 mg; Phenomenex, Torrance, CA, USA) conditioned with 3 ml of methanol and equilibrated with 2 ml of water, followed by 3 ml of 50 mM sodium phosphate (pH 10). The sample was loaded at 2 ml/min, and the cartridge was washed twice with 2 ml of water. The TA-aldol was eluted with 3 ml of 0.5% formic acid in methanol, and the eluate was evaporated to dryness and stored in a vacuum desiccator. The final product was assayed at 78% purity by HPLC and had a mass spectrum consistent with that of TA-aldol.

Soils

Six midwestern U.S. surface soils and one sandy surface soil from Florida (USA), representing a wide range in pH, percentage OC, CEC, and texture, were selected for the sorption studies (Table 1). Soils were air-dried, gently crushed, passed through a 2-mm sieve, and stored in air-tight containers at room temperature. Two additional midwestern surface soils, a silty clay loam (Drummer-30) and a sandy soil (Oakville-31), were collected from corn fields in April 2005 preharvest and December 2005 postharvest, respectively, and then passed through a 2-mm sieve and stored at 4°C before use. A subset of each soil was sterilized to differentiate between microbial and abiotic degradation. Sterilization was done by incubating moist soil for 3 d at room temperature, followed by irradiation with 1.15 MRad using a ^{60}Co irradiator (Gammacell 220; MDS Nordian, Ottawa, ON, Canada). The incubation and irradiation steps were repeated once, resulting in a total dose of 2.3 MRad.

Sorption experiments

Sorption isotherms were constructed using five concentrations of TA, TD, or TA-aldol ranging from 5 to 43 μmol/L in 0.01 N $CaCl_2$ in duplicate. Antibiotic solutions (35 ml) were added to 40-ml, glass centrifuge tubes equipped with Teflon®-lined screw caps containing 0.5, 2, or 4 g of soil (Table 1). Soil weights were selected based on preliminary studies to achieve 50 ± 30% sorption of the applied antibiotic. Experimental controls included antibiotic solution without soil and the 0.01 N $CaCl_2$ background electrolyte solution with soil. Tubes were wrapped in aluminum foil, rotated end-over-end for 60 h at 23 ± 3°C, and centrifuged at 630 *g* and 23 ± 3°C for 20 min (CR312, Jouan Scientific Benchtop Centrifuge; Thermo Fisher Scientific, Waltham, MA, USA). The supernatant pH was measured immediately after centrifugation, followed by sampling for analysis. The remaining supernatant was discarded and residual solution determined gravimetrically. Soils were extracted with 20 ml of methanol for 12 h on a rotary end-over-end mixer, centrifuged (630 *g* for 20 min), and sampled for analysis.

Aqueous solutions and solvent extracts of soils were analyzed with a HPLC (SCL-10A with LC-10AD pump and SIL-10A auto injector; Shimadzu, Kyoto, Japan) equipped with an ultraviolet–visible (UV) light detector (SPD-10A, λ = 280 nm)

and reverse-phase column (length, 150 mm; inner diameter, 4.6 mm; particle size, 5 μm; Supelcosil ABZ Plus; Supelco, Bellefonte, PA, USA). The mobile phase was 23:5:5:67 (v:v:v:v) acetonitrile/0.2 M tetrabutylammonium hydrogensulfate/0.2 M phosphoric acid/water at a flow rate of 1 ml/min. The injection volume was 25 μl. Concentrations were quantified using external standards prepared by serial dilution in methanol for the solvent extracts and in 0.01 N $CaCl_2$ for the aqueous solutions. Tylosin-A standards were used for TA-aldol, because quantities available from synthesis were limited and synthesis was time-consuming. The UV response factor for TA-aldol and TA were within 2% of each other.

Sorption isotherms were calculated using the Freundlich equation: $C_s = K_f C_w^N$, where C_s (mmol/kg soil) is the amount of antibiotic sorbed to the soil, C_w (mmol/L solution) is the aqueous equilibrium concentration, K_f (mmol^{1-N} L^N/kg) is the Freundlich sorption coefficient, and N (dimensionless) is a measure of sorption nonlinearity.

Degradation experiments

For a sandy soil [Oakville-31] and a silty clay loam (Drummer-30), degradation of TA was measured separately in tubes containing 5 g of moist soil. Water was added to achieve a 0.03 MPa moisture potential, and the soils were preincubated for 72 h. A 0.3-ml aliquot of aqueous TA solution (320 mg/L) was applied to soil to achieve an initial concentration of 20 mg/kg, and the samples were placed in a dark chamber to incubate at 23 ± 3°C. Samples were weighed daily, and water was added as needed to maintain a 0.03 MPa moisture potential. The tube closures were removed daily for 1 min to allow air exchange. After the designated incubation times, the samples were extracted with 30 ml, and again with 10 ml, of 70:30 (v:v) acetonitrile/0.3 M ammonium acetate, and the extracts were analyzed by liquid chromatography–mass spectrometry (LC/MS). Degradation experiments with irradiated soils were performed in a similar manner except that tubes were autoclaved for 1 h before soil addition and the tubes were not opened during incubation to reduce potential contamination with active microbes.

In a parallel experiment designed to optimize identification of degradates, double the tylosin mass was added (0.2 mg of tylosin per 5 g of soil). At 20 and 40 d, samples were extracted with 30 ml of 70:30 (v:v) acetonitrile/0.3 M ammonium acetate and assayed by LC/MS. Control samples were extracted in like manner immediately after application of tylosin to soil.

LC/MS conditions

Samples from the degradation studies were analyzed on an API 3000 mass spectrometer (Sciex, Concord, ON, Canada) coupled to a Shimadzu HTA liquid chromatographic system. Selected-ion monitoring (m/z = 916.5–917.5) was used for detection of TA in the samples. For determination of tylosin degradates, full-scan mode (m/z = 500–1,000) was used. Instrumental conditions were optimized while infusing a solution of 10 mg/L of TA into the eluent stream from the liquid chromatographic system. The mobile phase was 50:49.75:0.25 (v:v:v) methanol/1 mM aqueous NH_4OAc/formic acid at a flow rate of 0.25 ml/min. The column was a Phenomenex Luna C8(2) (length, 100 mm; inner diameter, 2 mm; particle size, 3 μm), and the injection volume was 10 μl.

RESULTS AND DISCUSSION

Sorption isotherms

A 95% ± 14% mass balance for TA, TD, and TA-aldol was obtained using methanol to extract soils except with the Toronto soil for which TA and TA-aldol recoveries were only 26 and 50%, respectively, as a result of solvent and pH-induced transformation. For TA extraction from Toronto soil, a chromatographic peak at a later retention time was noted and confirmed through LC/MS to have a mass 46 units higher than that of TA. In methanol under acidic conditions, TA can form TA dimethyl acetal, which is consistent with the mass determined and the conditions promoted by methanol extraction of a low-pH soil. This peak was not observed with either TA-aldol or TD, which lack the reactive aldehyde group. Tylosin recovery from the Toronto soil increased to 98% using 70:30 (v:v) acetonitrile/0.3 M NH_4OAc as the extractant, and the later eluting peak was not observed. For TA-aldol, a chromatographic peak appeared at a shorter retention time, which is hypothesized to be TB-aldol or, perhaps, a hydration product of TA-aldol (addition of water across one of the double bonds); however, confirmation by mass spectrometry was not attempted. Both are likely degradation products of TA-aldol and can be expected to elute before TA-aldol [14,32–34]. Additional post isotherm extraction tests with a single applied concentration showed that extraction with 70:30 (v:v) acetonitrile/0.3 M NH_4OAc improved recoveries to nearly 100% for most soil–solute combinations. For example, methanol extraction of tylosin from Drummer-1, which gave the lowest recovery for the soils in which no degradate was observed, increased from 85 to 107% using 70:30 (v:v) acetonitrile/0.3 M NH_4OAc. Although methanol extractions were good for the most part (95% ± 14%), it underestimated the amount sorbed in some cases or reacted with the analyte (e.g., low-pH Toronto soil); therefore, C_s was calculated from the equilibrium concentration by assuming that all tylosin lost from the initial equilibration solution was sorbed to soil.

Both TD and TA-aldol exhibit sorption characteristics similar to those of TA, as exemplified in the representative sorption isotherms shown in Figure 2 and the Freundlich isotherm fits summarized in Table 2. Sorption isotherms for most of the soils were nonlinear, with N values ranging from 0.4 to 1. Sorption nonlinearity suggests that more than one sorption domain contributes significantly to the sorption process. To evaluate the correlation between sorption magnitude and soil properties, concentration-specific distribution values (K_d^*) were estimated using the nonlinear sorption parameters ($K_d^* = K_f C_w^{N-1}$) at an equilibrium concentration of 2×10^{-4} mmol/L (Table 2), which falls within the measured C_w range for all but the Eustis soil. In general, the magnitude of tylosin and the related metabolites are positively correlated with clay (r^2 = 0.86) and OC content (r^2 = 0.41), surface area (r^2 = 0.91), and CEC (r^2 = 0.70) (Fig. 3).

It is difficult to assess the individual contribution to sorption of these domains given the direct relationship between CEC, OC clay content as well as clay content and surface area. We hypothesized that cation exchange would play a dominant role in the sorption of tylosin, because all the soils in the present study have a pH below the pK_a of tylosin (pK_a = 7.7). Recovery was enhanced using an extracting solution containing NH_4^+ to enhance exchange (acetonitrile/0.3 M NH_4OAc), and CEC-normalized, concentration-dependent sorption coefficients (K_d^*/CEC) fall within a narrow range ($10^{4.1\pm0.21}$ L/mol$_c$) with

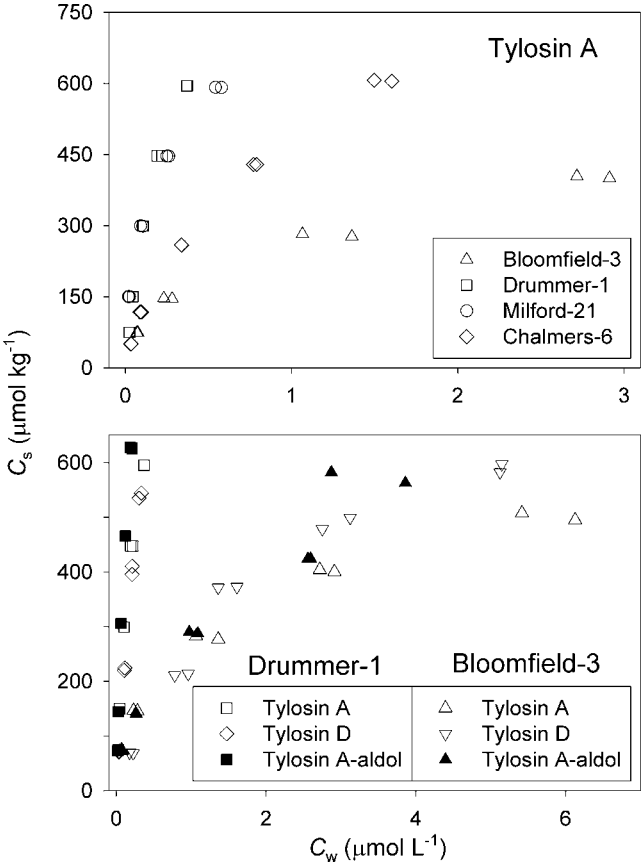

Fig. 2. Representative sorption isotherms for tylosin A on four soils (top) and comparison of tylosin A, tylosin D, and tylosin A-aldol sorption by two soils (bottom).

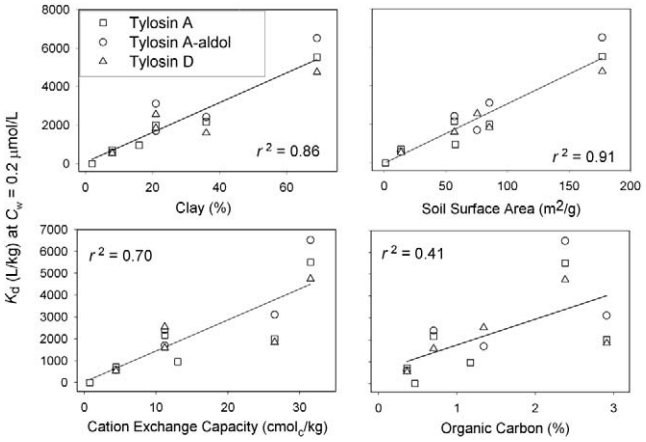

Fig. 3. Tylosin A, tylosin A-aldol, and tylosin D sorption coefficients calculated from the Freundlich isotherm model fits using a fixed aqueous-phase concentration of 0.0002 mmol/L plotted against clay content, soil surface area, cation-exchange capacity, and percentage organic carbon of soils.

the exclusion of the Eustis-25 soil, for which sorption was the lowest because of its low OC content and low CEC (Table 1). However, acceptable mass recovery was obtained for most of the soils with methanol, indicating that hydrophobic forces also are contributing significantly to the sorption process. In addition, we suspect that the large molecular size of tylosin may attenuate the interaction of the aliphatic amine group with surface exchange sites [26]. It also is probable that the tylosin molecule is precluded from entering into smectite interlayers because of its size, thus further reducing the potential for interaction with exchange sites. Kumar et al. [7] noted that whereas chlortetracycline (molecular wt, 528 g/mol) increased the interlayer spacing of clay minerals, the larger molecule tylosin (molecular wt, 916 g/mol) did not, which exemplifies tylosin's limited access to interlayer domains. Easier access to edge sites relative to interlayer sites also may be exemplified by the EPA-23 soil, which has the highest surface area, clay content, and CEC (Table 1), and the largest percentage of nonswelling clays (illite and kaolinite). Concentration-specific K_d values for reversible sorption by the EPA-23 soil were the highest observed in the present study and fall above the K_d-CEC correlation (Fig. 3), suggesting that the edge sites have a higher affinity than the interlayer sites for cation exchange.

In addition to limited access of tylosin and closely associated metabolites to interlayer exchange sites, hydrophobic interactions with siloxane surfaces and coordination bonding of tylosin carboxyl groups with surface-associated cations may play a significant role in tylosin sorption to soils [25,35], which could contribute to the relatively higher correlations observed with surface area and percentage clay versus CEC. No matter what the mechanism, soil clay content clearly has an influence on the degree of tylosin sorption, which can be utilized in the field to predict the potential for leaching of tylosin and related compounds following application of animal wastes.

Degradation experiment

A summary of the degradation of TA in two contrasting soils is shown in Figure 4. Based on the ^{60}Co-irradiated soil controls, it appears that both abiotic and microbial processes contribute to the transformation of TA. Loss of TA during the first two weeks is similar between the nonsterile soils and the

Table 2. Tylosin A and Tylosin A-aldol sorption coefficients and standard errors estimated from the Freundlich isotherm model fits

	Tylosin A			Tylosin D			Tylosin A-Aldol		
Soil	K_f[a]	N	K_d*[b]	K_f[a]	N	K_d*[b]	K_f[a]	N	K_d*[b]
Eustis-25	0.406 ± 0.082	0.80 ± 0.06	2.23						
Bloomfield-3	4.35 ± 0.51	0.40 ± 0.02	721	9.98 ± 3.00	0.53 ± 0.05	547	10.9 ± 3.5	0.53 ± 0.05	597
Chalmers-6	27.1 ± 3.4	0.58 ± 0.02	969						
Milford-21	15.5 ± 2.6	0.42 ± 0.02	2,170	124 ± 64	0.70 ± 0.07	1,600	73.7 ± 25.9	0.59 ± 0.04	2,420
Drummer-1	132 ± 53	0.68 ± 0.05	2,010	310 ± 91	0.79 ± 0.04	1,850	340 ± 159	0.74 ± 0.05	3,110
EPA-23	168 ± 25	0.59 ± 0.02	5,520	262 ± 28	0.66 ± 0.02	4,745	465 ± 319	0.69 ± 0.09	6,520
Toronto-4	C_w < LOD[c]			2,560 ± 217	1	2,560	185 ± 83	0.74 ± .05	1,699

[a] mmol^{1-N} L^N/kg.
[b] Concentration-specific distribution coefficient (L/kg) calculated from Freundlich model fit at $C_w = 2 \times 10^{-4}$ mmol/L.
[c] LOD = Limit of detection.

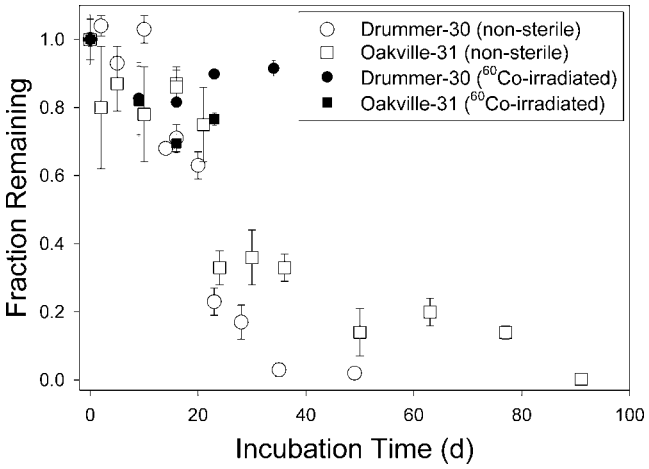

Fig. 4. Degradation (mass remaining of applied over time) of tylosin A in nonsterile (open symbols) and ^{60}Co-irradiated (closed symbols) Drummer-30 (circles) and Oakville-31 (squares) soils.

soils sterilized with ^{60}Co irradiation, suggesting that abiotic processes may dominate at early times; however, confirmation of the complete absence of active microbes was not done. In any case, the microbial consortium appeared to need nearly three weeks to adapt to TA degradation, but once initiated, more than half the compound was degraded within 3 d (between days 20–23 and 21–24 for the Drummer-30 and Oakville-31 soils, respectively). These half-lives of just over three weeks in soils, which includes the long lag time, are shorter than what was observed for TA in manure (200 mg/kg dry wt) applied to fields of sandy loam (half-life, 54–86 d) and sandy soils (half-life, 40–64 d) [20].

Identification of tylosin degradates

A degradate with m/z = 932 was found in both Drummer-30 and Oakville-31 soils. This peak, which was 50-fold smaller than the TA peak in the day 0 control samples, increased by a factor of nine at day 20 and by a factor of 10 at day 40 in the Drummer-30 soil. In the Oakville-31 soil, the peak area for this compound increased by a factor of eight at day 20 and by a factor of 14 at day 40. This degradate most likely is TA with the aldehyde group oxidized to an acid (TA-acid). By day 40, the TA-acid peak was approximately one-third the size of the TA peak in the Oakville-31 soil and one-fifth the size of the TA peak in the Drummer-30 soil. Because the aldehyde group appears to be particularly important for the antimicrobial activity of tylosin, it may be that this compound is significantly less active than TA. Other possible positions of transformation that would result in a degradate with m/z = 932 include conversion of the ketone to an ester or addition of oxygen to one of the double bonds. Both of these transformations are not likely to reduce the antimicrobial potency of the parent compound.

Two other degradates with m/z = 772 and 788 were found in significant quantities in the Oakville-31 soil. In the day 0 control samples, the peak area for the m/z = 772 peak was 3% as large as the area for TA, whereas the m/z = 788 peak was not detected in the controls. These compounds, which were observed in both the day-20 and day-40 samples, most likely are TB and TB with the aldehyde group oxidized to an acid (TB-acid). The TB peak area increased by a factor of 8.5 during 40 d of incubation and was more than one-fourth the area of the TA peak by day 40, at which time approximately 30% of the applied TA remained. The TB-acid peak at day 40 was approximately 10% of the TA peak area. As noted previously, TB retains much of the antimicrobial potency of TA, whereas TB-acid may be expected to have a significantly reduced potency.

CONCLUSIONS

Tylosin and primary degradates exhibit similar sorption characteristics and are strongly sorbed to most soils studied, with sorption highly correlated to surface area, clay content, and CEC. Sorption coefficients normalized by cation-exchange capacity were within a narrow range ($10^{4.1\pm0.21}$ L/mol$_c$) for all but one soil; however, good extraction recoveries with only methanol suggested that hydrophobic processes also contribute to sorption. Aerobic degradation of TA over a three-month period in two freshly collected agricultural soils and ^{60}Co-irradiated soils indicated that both abiotic and microbial processes contribute to TA transformation. The abiotic process was much slower and dominated in the first few weeks, followed by rapid microbial degradation within a few days. One primary degradation product, which appeared to be from oxidation of the aldehyde, was tentatively identified as TA-acid (m/z = 932) in both soils. Two additional primary degradation products were identified in the sandy soil, which were hypothesized to be the TB (m/z = 772) and TB-acid (m/z = 788) compounds. Tylosin B is known to have antimicrobial potency similar to that of TA [9]; antimicrobial activity of the metabolites have not been confirmed. In any case, the strong sorption of TA and its metabolites to soils will mitigate transport of these antibiotics, which will help to retain them in the top soil and to enhance the residence time needed for degradation to occur. Based on these findings, it can be inferred that transport of TA to groundwater and/or surface waters following field application of manure from waste lagoons is not likely to be a concern except in cases when a significant rain event occurs immediately following manure application.

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