Soil & Tillage Research 158 (2016) 57–66

Contents lists available at ScienceDirect

Soil & Tillage Research

journal homepage: www.elsevier.com/locate/still

Earthworm epidermal mucus: Rheological behavior reveals drag-reducing characteristics in soil

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ARTICLE INFO

Article history:
Received 3 February 2015
Received in revised form 26 November 2015
Accepted 10 December 2015
Available online 28 December 2015

Keywords:
Viscosity
Fluids
Bionic
Adhesion reduction

ABSTRACT

Soil adhesion occurs on the surfaces of soil-tillage implements during use. This phenomenon increases energy consumption and decreases tillage quality. Nevertheless, earthworms can comfortably move in moist or adhesive soil, although with soil particles seldom sticking to bodies. The influence of earthworm movement on epidermal mucus provides perspective for the interpretation of the results on drag-reducing characteristics. The amino acid composition and rheological behavior of epidermal mucus samples from three earthworm ecological species were studied in the laboratory. These earthworm species were represented by *Eisenia fetida*, *Aporrectodea trapezoides*, and *Amynthas pingi*, which were collected from the Typic Hapludolls with loam textures. An electro-stimulation device was developed to extract and collect approximately 5 g of mucus (field weight) from several individuals of 30 g in total of each earthworm species. Mucus samples from the three earthworm species were found to contain 16 amino acids. Aspartic acid, glutamic acid, and valine were present in relatively high concentrations in all the samples. The rheological behavior of earthworm epidermal mucus was investigated via continuous shear, thixotropy, and oscillation tests. The apparent viscosity initially increased and then decreased with the increase in mucus shear rate. The thixotropic behavior was demonstrated as the apparent viscosity of epidermal mucus recovered gradually with the decrease in shear rate. Oscillatory tests showed that the viscoelastic behavior of epidermal mucus can be altered with changes in oscillation frequency. A five-layer interface contact model revealed that the apparent viscosity of mucus changes with earthworm movement, thereby reducing the soil adhesion and friction resistance toward the earthworm body surface. Overall, this study revealed the drag-reducing characteristics of earthworm epidermal mucus and inspired the development of some potential bionic applications with lubricating functions for soil-tillage implements.

1. Introduction

The interactions of living organisms with natural surroundings have led to the evolution of biological systems and environmental adaptabilities. Earthworms have long been acknowledged to largely contribute to the aggregate stability of soils varying in texture, carbonate, and concentration of organic matter by burrowing, foraging, and casting on the soil surface and within the soil (Fonte et al., 2012; Laossi et al., 2010; Pulido Moncada et al., 2014). Earthworms are typical soil-burrowing animals (Grdisa et al., 2013; Ren et al., 2001a; Struck et al., 2011) and can be classified into three ecological groups, namely: epigeics (litter dwellers; shallow horizontal burrows), endogeics (soil dwellers, netlike burrow system) and anecics (litter feeders and soil dwellers, deep vertical burrows) (Bouché, 1977). Epigeics are small, reddish, and quick-moving earthworms with short life cycles. Endogeics are slow-moving earthworms with variable sizes and intermediate longevity. Anecics are large, dark-colored earthworms that can rapidly withdraw but slowly move forward (Asshoff et al., 2010; Palm et al., 2013; Scullion et al., 2007).

Earthworms can readily move in moist or adhesive soil with soil particles seldom adhering to bodies. Earthworms are slippery when held, which can be mainly attributed to the layer of epidermal mucus enveloping the body surface, similar to fish slimes (Rosen and Cornford, 1971). Meanwhile, the layer of epidermal mucus serves as an important protective barrier for earthworms. Earthworm mucus contains cells and many other molecular components involved in innate immunity. Mucus

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http://dx.doi.org/10.1016/j.still.2015.12.001

proteins comprise the basic components of innate immunity in earthworms (Kauschke et al., 2007). The epidermal mucus layer forms and maintains an interface with dissolved oxygen for gas exchange; it protects and lubricates the epithelium and other surfaces, providing first-line defense against invading pathogens (Heredia et al., 2008; Wang et al., 2011). Furthermore, epidermal earthworm mucus buffers the pH of the burrow wall (Schrader, 1994). Earthworms uphold metabolic balance by mucus osmoregulation. Various organic matter in mucus serve as nutrients for plants and enhance the decomposition activities of soil microbes (Marichal et al., 2011). Either earthworm mucus or amino acids significantly promoted plants growth (Zhang et al., 2009), implying that earthworm epidermal mucus could serve as a kind of amino acid liquid fertilizer which provide nutrients to plants.

Earthworm epidermal mucus is widely believed to play an important role in reducing soil adhesion and friction resistance during earthworm locomotion, burrowing, and feeding in soil. Effective energy-saving characteristics of soil-burrowing animals, including earthworms, are usually studied by agricultural engineers. However, the drag-reducing mechanisms in earthworms remain unclear. Notably, Chen et al. (1990) and Ren et al. (1990) observed and examined the geometrical surface morphologies of earthworms. Their findings showed that earthworms can decrease the soil friction resistance by secreting body fluids that infiltrate into the soil layer as the organisms move through the soil. These fluids serve as lubricants and thus reduce soil adhesion to the earthworm body surfaces. Saha and Celata (2011) discussed the electro-osmotic effects of non-smooth surfaces. A layer of water film is formed from the migration of water to the earthworm body surfaces, which improves the efficiency of reducing soil adhesion to the surfaces. Li et al. (2010) observed the non-smooth surface morphology of earthworm specimens and designed similar bionic samples. The test results showed that the soil resistance of the bionic samples decreased by 76.8% compared with that of the non-bionic ones. As demonstrated by these studies, earthworm mucus promotes the reduction of soil adhesion and resistance. However, few studies have focused on the flow behavior and drag-reducing characteristics of earthworm epidermal mucus.

Earthworms naturally secrete small volumes of epidermal mucus while moving through the soil (Li et al., 1990). Natural collection are less harmful to the live earthworms, and the stable epidermal mucus composition can be obtained during the collection process. However, a long period is required to gather sufficient mucus volumes, which is further complicated by the easy evaporation of mucus to the air (Jiang et al., 1990). Hence, earthworm epidermal mucus is difficult to extract and collect. The low-voltage electro-stimulation method can be used for collecting the earthworm epidermal mucus efficiently (Liu et al., 2004; Wang et al., 2007a).

The current work aims to (1) develop an extraction device to collect earthworm mucus, (2) analyze the rheological behavior of mucus samples, and (3) elucidate the drag-reducing mechanisms of earthworm mucus. Soil adhesion occurs on the surfaces of soil-tillage implements during their use. The findings of this study are expected to provide basis for the design of bionic soil-tillage implements with decreased drag resistance, reduced energy consumption, and improved working quality.

2. Materials and methods

2.1. Sample collection

The epigeic species *Eisenia fetida*, the endogeic species *Aporrectodea trapezoides*, and the anecic species *Amynthas pingi* were selected for this study because of different ecological habits in soil. Therefore, the mucus samples obtained from three earthworm species are suitable for different soil environments. These three earthworm species are widely distributed in China. In particular, *E. fetida* has been commonly fed and used in laboratory experiments, whereas *A. trapezoides* and *A. pingi* are common species widely spread in permanent farm or pasture soil (Lowe and Butt, 2005; Topoliantz et al., 2000). Hence, these earthworms can be collected without compromising the survival of their species. Samples of live *E. fetida* were collected from an earthworm farm (43°52′N, 125°19′E), *A. trapezoides* from a corn farm (43°50′N, 125°18′E), and *A. pingi* from the Jingyuetan National Forest Park in the suburb of Changchun City, Jilin Province, China (43°47′N, 125°27′E).

Table 1
Particle size distribution of the soil from the collection sites of three earthworm species.[a]

Soil samples	Particle size distribution ($g\,kg^{-1}$)		
	Clay (<0.002 mm)	Silt (0.05–0.002 mm)	Sand (2–0.05 mm)
E. fetida	101.89 ± 5.52	393.17 ± 6.95	504.94 ± 9.02
A. trapezoides	243.84 ± 4.72	407.45 ± 6.85	348.71 ± 9.34
A. pingi	125.54 ± 5.16	427.16 ± 5.45	447.30 ± 6.73

[a] Values represent the mean ± standarddeviation of three measurements.

According to the principles of soil sample preparation and particle-size analysis methods (Gee et al., 1986). Soil samples were collected using a soil auger from the surface to a 20 cm depth in each species collection site. The soil samples were air-dried, and the clods were smashed with a wooden mallet. Tree roots and other debris were removed from the samples. The soil samples were sieved through 2.0, 0.6, 0.355, and 0.075 mm soil sieves. The soil retained on each sieve was weighed, and the particle sizes (<0.075 mm) were determined by a laser particle-size analyzer (BT-9300ST, Dandong, China). Based on the USDA soil texture classification (USDA, 2014), the particle size distribution of soil is given in Table 1. The soil properties sampled from the collection site of earthworms are the Typic Hapludolls (USDA, 2014; Zhang et al., 2015) with loam textures.

The collected earthworms were washed with distilled water and dried using filter papers. Mature clitellate individuals were selected and weighed. The epidermal mucus of earthworm was recorded using a stereomicroscope (SteREO Discovery V12, Carl Zeiss), as shown in Fig. 1.

2.2. Electro-stimulation device and collection of earthworm epidermal mucus

An electro-stimulation device was designed to extract and collect earthworm epidermal mucus. An adjustable DC power source (output voltage of 3–12 V) is connected to binding posts through alligator clips and wires, which would supply power to the guide rails. A plate electrode comprising metal plate and an insulated handle is placed on the rails. After switching the power on, the metal plate in contact with the guide rail is charged while the handle is being insulated from the guide rail on the other end. Particularly, a single-plate electrode is charged either positively or negatively. Two adjacent electrodes with opposite polarities comprise the electrode group, and the distance between the two electrodes can be adjusted. As the earthworms move into the electrode groups of the extraction box, the positive and negative electrodes would be connected by the earthworm bodies. A current loop would then be produced to electro-stimulate the earthworms, and consequently they secreted mucus rapidly. At such time, the secreted epidermal mucus was dripping into the collection box through a separation plate with cone-shaped holes.