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Earthworm gut bacteria increase silicon bioavailability and acquisition by maize

Lin Hu[a,b], Ming Xia[b], Xianhui Lin[b], Cuicui Xu[b], Wu Li[c], Jie Wang[a], Rensen Zeng[a,**], Yuanyuan Song[a,*]

[a] Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, College of Crop Science, Fujian Agriculture and Forestry University, Fuzhou, 350002, China
[b] College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, 350002, China
[c] Crops Research Institute, Guangdong Academy of Agricultural Sciences, Guangdong Provincial Key Laboratory of Crops Genetics Improvement, Guangzhou, 510640, China

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ABSTRACT

Silicon improves plant resistance to a wide range of biotic and abiotic stresses. However, available silicon as a plant nutrient in soil is scarce. A continuous supply of available silicon is vital for plants. Earthworms are important detritivores in many terrestrial ecosystems. Therefore, we hypothesized that earthworms harbor silicate solubilizing bacteria in their guts and play a key role in silicon weathering and dynamics. Silicate solubilizing bacteria were isolated from the gut of geophagous endogeic earthworm (*Pheretima guillelmi*) and their effects on silicon weathering and availability to maize (*Zea mays*) were examined. Significantly more silicate solubilizing bacteria were observed in the earthworms gut than those in the surrounding soil in all sampling locations. The 16S rRNA analysis showed a higher silicate solubilizing bacteria diversity with 20 bacterial strains isolated from the gut walls, belonging to the genera of *Aeromonas, Bacillus*, *Cellvibrio*, *Ensifer*, *Flavobacterium, Microbacterium*, *Paracoccus*, *Pseudomonas*, *Rhizobium,* and *Streptomyces*. Of these bacteria, only three species were found in the surrounding soil. Inoculation with the three fastest-growing strains in liquid culture increased soluble silicon released from the feldspar and quartz powder in the media. Inoculation with the 3C1 strain in the potting soil significantly increased soluble silicon content in the soil, enhanced silicon uptake and accumulation in maize plants and promoted seedling growth. Taken together, these results not only highlight the importance of earthworm gut microbes in soil silicon weathering but also indicate a new approach in searching for silicate solubilizing bacteria to increase soil silicon availability in agriculture.

1. Introduction

Silicon (Si) is the second most abundant element after oxygen in the soil and the Earth's crust. Although it has not been recognized as an essential element in plant physiology (Epstein, 1994), Si has been widely accepted as a beneficial element in agriculture, and it is receiving increasing attention because of its multiple functions in plant stress resistance (Liang et al., 2015; Reynolds et al., 2016). Si not only boosts plant growth but also enhances plant resistance to a large range of biotic and abiotic stresses including herbivores, pathogens, salinity, heavy metal toxicity, drought, radiation damage, nutrient imbalance, and extreme temperatures (Epstein, 1999; Ma, 2004). Importantly, the most important food crops, such as rice, maize, wheat, banana, and sugarcane, are Si-accumulating plants (Liang et al., 2015). Si accumulation can reach 10% in rice shoots (Ma and Takahashi, 2002).

Soil Si is plentiful and usually ranges from 25% to 35%, depending on soil types. It exists primarily in oxide or silicate forms in minerals and rocks, which are water-insoluble and cannot be directly absorbed and assimilated by plants (Richmond and Sussman, 2003). Continuous crop harvest results in the removal of a large amount of available Si from croplands, particularly after cultivation of these Si-accumulating monocotyledon crops (Liang et al., 2015). For example, the cultivation of sugar cane and rice removes Si by 300 kg/ha/year and 500 kg/ha/year, respectively (Makabe et al., 2009; Meyer and Keeping, 2001). In agriculture, a large amount of nitrogen (N), phosphorus (P) and potassium (K) fertilizers have been used worldwide, however, utilization

* Corresponding author.
** Corresponding author.
E-mail addresses: rszeng@fafu.edu.cn (R. Zeng), yyuansong@163.com (Y. Song).

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of Si fertilizer is often ignored. Therefore, available Si as a plant nutrient in soils is frequently limited and it is important to convert unavailable Si into available form in the soils for sustaining high production of crops.

Microorganisms play a fundamental role in soil weathering by dissolving nutrients from insoluble minerals (Uroz et al., 2009). Microorganisms directly or indirectly activate mineral physical and chemical changes by excreting organic compounds on the mineral surface (Banfield et al., 1999). In the 18th century, microbes were used to extract metals from ores in the mining industry (Rawlings, 2002). Soil microbes have long been recognized as a source of biological fertilizer (Vessey, 2003). The application of microbial fertilizer not only decreases the economic cost but also reduces the environmental pollution. Certain silicate solubilizing bacteria (SSB) have been demonstrated to be able to improve the quality and purity of ore by removing Si (Karavaiko et al., 1980; Smith, 2009). SSB can solubilize Si from silicate bearing mineral and rocks (Bosecker, 1997). These bacteria also increase the dissolution of diatom silica in the ocean (Bidle and Azam, 1999). Therefore, screening for effective Si-solubilizing microbes is a promising approach to increase Si bioavailability, crop yield and resistance in Si-deficient soil.

Soil animals, such as earthworms, beetles and termites, are key mediators of soil formation and functions (Jouquet et al., 2002; Suzuki et al., 2003). Earthworms are keystone macro-invertebrate detritivores that play a key role in soil fertility, nutrient cycling and plant growth through earthworm-microorganism interactions in many terrestrial ecosystems (Edwards and Bohlen, 1996; Thakuria et al., 2010; Winding et al., 1997). These worms exert strong impact on the soil microbial community (Braga et al., 2016; Dempsey et al., 2013; Groffman et al., 2015; Lin et al., 2016). When the soil passes through the earthworm gut, it undergoes many physical, chemical and microbial changes (Zhang and Schrader, 1993). The amounts of bacteria in the casts are considerably higher than that in the surrounding soil (Jirout and Pižl, 2014). However, the bacterial composition in earthworm burrowed soils and guts appears to be highly similar (Satchell, 1983). Hence, the earthworm gut microbes play an active role in soil genesis (Drake and Horn, 2007). High microbial activities enhance the carbon (C), N, P cycling in the drilosphere (Hoang et al., 2016). Increasing evidence shows that earthworm gut microorganisms are involved in soil weathering. Recently, P and K solubilizing bacteria have been isolated from earthworm gut (Liu et al., 2016; Prasanna et al., 2010).

Bityutskii et al. (2016) reported that earthworms increase water solubility and bioavailability of Si in various types of soils, but the underlying mechanism has not been thoroughly elucidated to date. Therefore, we hypothesized that geophagous endogeic earthworm gut harbors microorganisms that participate in soil Si weathering and dissolution and thus increase Si bioavailability.

The objectives of this study were (1) to isolate and identify Si-solubilizing bacteria from earthworm gut, (2) to assess the weathering ability of selected bacteria, and (3) to examine the effect of soil inoculation of selected bacteria on Si availability in the soil and Si uptake in maize plants.

2. Materials and methods

2.1. Earthworms, plant, soil and mineral

Individuals of earthworm *Pheretima guillelmi* Michaelsen were collected from roadside topsoil in the Experimental and Teaching Farm of Fujian Agriculture and Forestry University, Fuzhou, China (119º54′ E, 26º05′ N) in May 2016. *P. guillelmi* is an endogeic earthworm species, which inhabits the mineral soil horizons and consumes soil according to the classification of Bouché (1977). The roadside was covered by indigenous wild herbaceous plants including *Cynodon dactylon* (L.) Pers., *Galinsoga parviflora* Cav., *Viola inconspicua* Bl., and *Hedyotis diffusa* Willd. Adult earthworms and surrounding soil were obtained from 10 random sampling locations. Surrounding soil samples were collected to avoid earthworm burrows and roots from a depth of 5–10 cm below the litter layer. The collected earthworms and soil were maintained in separate plastic containers in the laboratory until the isolation experiment began in the following day. The remaining samples were kept at 4 °C.

To compare the abundance of SSB in earthworm gut and surrounding soil, earthworms and soil were sampled from the six locations described above in March 2018. In each location, sampling was repeated 3 times.

Seeds of maize (*Zea mays* L. cv. Yuebai) were supplied by Crops Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China.

The local red soils for maize pot culture were collected from an abandoned hillside near the main campus of Fujian Agriculture and Forestry University. The air-dry soil was screened to pass a 2-mm mesh stainless steel screen for planting maize seedlings. The chemical properties of the soil were: pH (w:v water of 1:2.5) 5.50; organic C 10.03 g kg^{-1}; total N 1.28 g kg^{-1}; available N 19.30 mg kg^{-1}; total P 0.13 g kg^{-1}; available P 12.60 mg kg^{-1}; total K 2.05 g kg^{-1}; available K 154.60 mg kg^{-1}, water-extractable Si 34.26 mg kg^{-1}.

Si bearing mineral powders including feldspar ($KAlSi_3O_8$, SiO_2 68.09%) and quartz (SiO_2 98.81%) were purchased from Foshan Hongda Ceramics Co., Ltd and passed through 75-μm mesh sieve. The powders were soaked in pure water for 3 days, and the water was changed daily to exclude soluble Si.

2.2. Isolation of SSB from earthworm gut wall and surrounding soil

Earthworm gut walls were isolated as previously described (Singleton et al., 2003; Thakuria et al., 2009), with minor modifications. Fifteen collected adult earthworms were cleaned with 70% ethanol for 30 s to remove surface bacteria. Then, the immovable earthworms were rinsed twice with sterilized water. The gut walls were opened to facilitate the removal of the ingested soil. A sterile stainless-steel spoon was scraped along the gut intestine to eliminate soil material physically. Thereafter, the gut content of earthworm was removed by washing the intestines with phosphate buffered saline (pH 7.2) five times. The washed intestine was used as gut wall sample. The gut wall samples were transferred into 15 mL sterile tube with sterile distilled water, sonicated for 1 min, and shaken at 180 rpm for 15 min at 28 °C. The mixture was allowed to stand for 30 min at room temperature. Surrounding soil homogenates were prepared in the same way as gut homogenates, except dissection and washing. The suspension was diluted and spread on the surface of Aleksandrov's medium for SSB culture containing 0.5% sucrose, 0.2% Na_2HPO_4, 0.05% $MgSO_4{\cdot}7H_2O$, 0.5% $FeCl_3$, 0.01% $CaCO_3$, 0.1% $KAlSi_3O_8$, 2% agar, pH 7.2. The Petri-dishes were placed in an incubator at 28 °C for 5 d. Bacterial growth was monitored every day. Fast-growing isolates with different morphology were selected for sub-culturing (Xu et al., 2015). The pure colonies were inoculated into the liquid medium and preserved in 25% glycerol solution at −80 °C.

2.3. Enumeration of SSB in earthworm gut and surrounding soil

To examine the abundance of SSB in earthworm guts (wall plus content) and surrounding soil, guts and soils collected from 6 sampling locations were weighed and homogenized as described above. Additionally, the remaining samples were oven-dried at 105 °C for 48 h for gravimetric determination of sample water. For counting the number of SSB, gut and soil homogenates were serially diluted (1:10) in sterile distilled water. Dilutions of each material were plated on Aleksandrov's plates. All plates were incubated at 28 °C for 48 h. The total SBB numbers were then estimated by counting the number of colony-forming units (CFU) per gram dry weight.