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Review

# *Bradyrhizobium japonicum* USDA110: A representative model organism for studying the impact of pollutants on soil microbiota

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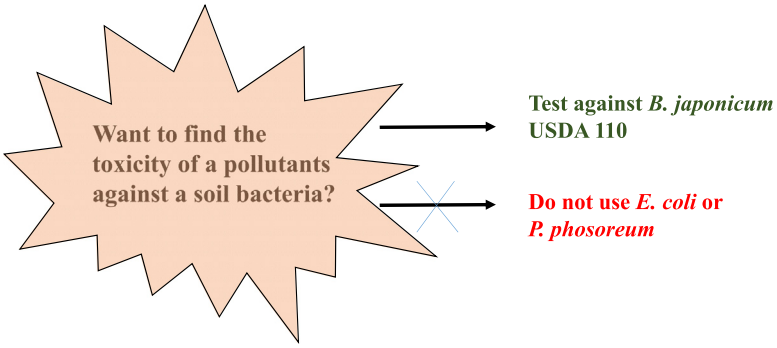
## HIGHLIGHTS

- Soil environment is dominated by Gram-negative organisms.
- Bacteria from Bradyrhizobium genus are the most ubiquitous organisms in the soil.
- *Bradyrhizobium japonicum* USDA110 should be used for toxicity screening of pollutants against soil microbial community.

## GRAPHICAL ABSTRACT

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## ABSTRACT

*Photobacteria phosoreum* or *Escherichia coli* are widely used in the scientific, industrial, and regulatory industries for evaluating the toxicity of pollutants against the soil microbial community. The organisms, however, are not part of the soil microbiota and the toxicity data obtained using these organisms could be misleading. Analysis of microbiota present in the soil obtained from across the world indicates that organisms from the *Bradyrhizobium* genus are the most ubiquitous of all microorganisms. Playing a critical role in nitrogen fixation and soil fertility, organisms from this genus should be used for studying the toxicity of pollutants. Indeed, we propose that *Bradyrhizobium japonicum* USDA110 be used as a model organism for screening pollutants for toxicity against a soil microbial community.



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Microorganisms present in the soil play an important role in maintaining the overall integrity of soil (Shah et al., 2011). They are vital for soil fertility, organic matter decomposition, nutrient provision for plant growth, and pollutant degradation (Garbeva et al., 2004; Van Beelen and Doelman, 1997). Perturbation of the composition of soil microbiota could have significant implications in the functioning of an ecosystem. This concern is more important considering the lack of our understanding of how the diverse array of anthropogenic pollutants is impacting the soil microbial diversity and a general lack of knowledge of the resulting effects, if any, at local, regional, and global scales (Giller et al., 1998). Review of the methods used to assess the impact of pollutants on soil microbiota indicates, that in spite of advances in -omics disciplines, the approved methods have not been modified to overcome the limitations of each method.

Related to the current study, *Photobacterium phosoreum* is suggested as the model test bacteria to rapidly evaluate the toxicity of pollutants to microbiota in soil (Van Beelen and Doelman, 1997; Hooper, 2008). Microtox™, a bioluminescence test involving *P. phosoreum*, is widely used to detect the level and degree of toxicity of pollutants. An increase in toxicity is directly correlated to a decrease in light output (Ribo and Kaiser, 1987; Markwiese et al., 2001). Another commonly used bacteria to screen for eco-toxicity of pollutants is *E. coli*. This organism is easy to handle in the lab and a decrease in its optical density or colony forming units allows scientists to compare the level of toxicity of different pollutants (e.g. Rispoli et al., 2010; Jiang et al., 2009). Literature review shows eco-toxicological studies of pollutants with other test microorganisms as well (e.g. Evans et al., 1998; Boyd et al., 1997; Ronnpagel et al., 1995).

A major drawback of using *P. phosoreum*, *E. coli*, or similar organisms is that none of these organisms are integral components of soil microbiota or occupy an ecologically important niche in the ecosystem. *P. phosoreum* is found in an aquatic habitat and *E. coli* is associated with the digestive system of animals. Conclusions reached from eco-toxicity experiments utilizing these organisms are often far-reaching and over-generalized. They are based on the assumption that toxicity studies against these organisms would most likely indicate whether a contaminant is toxic against ecologically important microbes in the soil. To overcome the disadvantages of such conclusions, a target organism needs to be identified that is an important part of the soil microbiota and performs a key ecological function.

With the rapid advancement of community based sequencing methods, scientific literature now exists on the composition of total microbiota in soil. The goal of the current study is to use the soil microbiota data generated using the 16S rDNA PCR-sequencing method and to identify a bacteria that could be used for eco-toxicological studies of pollutants on microbiota present in the soil matrix.

Top soil samples used in the study were obtained from 13 property owners and public locations at various locations in the continental USA and immediately sealed in plastic bags. No permits were required for collection. Upon receipt, visible debris was removed from the soil samples and microbiota determined using the methodology described in our earlier study (Shah et al., 2016). In brief, the soil samples were first treated with ethidium monoazide (EMA) (Pisz et al., 2007). DNA was extracted from the soil using PowerSoil™ DNA isolation kits (MO BIO Laboratories Inc., Carlsbad, CA). Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) were performed as described previously using Gray28F 5′ TTTGATCNTGGCTCAG and Gray519r 5′ GTNTTA CNGCGGCKGCTG primers (Omer et al., 2012). Amplicons originating and extending from the primers were used for the initial generation of the sequencing library. Pyrosequencing analyses utilized the Roche 454 FLX instrument with Titanium reagents and Titanium procedures and were performed at the Research and Testing Laboratory (Lubbock, TX) based upon RTL protocols (www.researchandtesting.com). After sequencing, all failed sequence reads, low quality sequence ends, tags and primers as well as any non-bacterial ribosome sequences and chimeras were removed using the UCHIME chimera detection software in de novo mode (Edgar et al., 2011). Short (<150 bp) reads were also removed. To determine the identity of bacteria in the remaining sequences, sequences were denoised, assembled into OTU clusters (97% identity), and globally aligned against a database of high quality 16S rRNA bacterial gene sequences compiled by RTL to determine taxonomic classifications (Shah et al., 2016).

To obtain further microbiota data already reported, a literature search was conducted by finding published papers from 2005 on the database, Web of Science, under the keywords "bacterial community soil," "soil microbial flora," and "soil bacterial flora". Papers were filtered by the parameters of having the microbiota obtained using the16S rDNA PCR-sequencing method, described at the genus level. 11 studies were found that fit these parameters and a total of 39 samples were found to be reporting microbiota from original soil in these studies (Acosta-Martínez et al., 2008; Fulthorpe et al., 2008; Shah et al., 2014; Zhalnina et al., 2013; Li et al., 2012; An et al., 2013; Kim et al., 2014a; Joa et al., 2014; Kim et al., 2014b; Sun et al., 2014; Nie et al., 2012). The data provided in each of the studies were tabulated as the percent abundance of the soil microbiome at the genus level. In total, 52 different soil microbiota were compared. Table S1 provides the complete microbial diversity within the soil samples at the genus level.

Table 1 shows the top 20 most abundant genera found in the soil samples along with the heat-map representing the relative percentage of each genera within each soil sample. Results indicate that Gram-negative organisms are predominant organisms present in the soil, with 16 of the top 20 genera being Gram-negative. Kaiser and Benner (2008) reported that the marine environment is dominated by gram negative organisms (Kaiser and Benner, 2008). Data in Table 1 suggests that similar to a marine environment, the soil environment is dominated by Gram negative organisms. *Streptomyces*, *Rubrobacter* and *Mycobacterium*, all members of Actinobacteria phylum, were the Gram-positive organisms found in the soil microbiota along with organisms belonging to the *Bacillus* genus.

Microorganisms playing critical roles in the nitrogen cycle are the most abundant bacteria in the soil at the genus level (Table 1). They include organisms from *Bradyrhizobium*, *Sphingomonas*, *Flavobacterium*, *Pontibacter*, and *Nitrosovibrio* genus. The nitrogen fixation and ammonia oxidation processes carried out by these organisms play a critical role in maintaining the fertility of soil. Of these organisms, *Bradyrhizobium* was found in excess of 0.5% in 27 of the 52 microbiota compared with values ranging from 0.6% to 11.3%, with an average of 3.0%. Heat-map analysis indicates that no other organism is more ubiquitous than *Bradyrhizobium*. *Burkholderia* is the second most prevalent genera, present in excess of 0.5% in 20 of the 52 microbiota analyzed.

Based on its prevalence and the key ecological functions they perform, we propose that organisms from the *Bradyrhizobium* genus be used for studying the eco-toxicity of pollutants on soil microorganisms. Numerous studies in literature validate the ubiquity of *Bradyrhizobium* in soil (Tan et al., 2001; Mathis and McMillin, 1996a; Vinuesa et al., 1998; Ormeño-Orrillo et al., 2012; Ozawa and Yamaguchi, 1986; Florentino et al., 2010; Moreira et al., 2006; VanInsberghe et al., 2015). Beyond agricultural soil, the organisms have been detected in forest soils and include non-symbiotic species (Ormeño-Orrillo et al., 2012; Moreira et al., 2006; VanInsberghe et al., 2015). *Bradyrhizobium* has also been used as a model organism to study the impact of chemical contaminants and physical stressors on soil microorganisms. The surveyed literature illustrating the use of organisms to measure toxicity include chlorimuron-ethyl (Zawoznik and Tomaro, 2005), heavy metals (Reichman, 2014; Keyser and Munns, 1979), metal-rich sewage sludge (Kinkle et al., 1987), acidity (Keyser and Munns, 1979), phospate (Keyser and Munns, 1979), herbicides (Moorman, 1986), osmotic stress (Soria et al., 2006) and, nanoparticles (Embleton, 2016) among the stressors studied. Taken together, the literature suggests that the selection of *Bradyrhizobium* for eco-toxicity studies is scientifically valid.

There needs to be a singular, established model organism that could be used by all members of the scientific community to perform eco-toxicity tests. Only then can there be a proper comparison of eco-toxicity