Effects of Nanoparticles on Double-Stranded RNA Stability in Corn Soil

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Abstract

Double-stranded RNA (dsRNA) can potentially be used as a pesticide because these molecules trigger an immune response called RNA interference (RNAi). If the expression of essential genes matching the dsRNA sequence are silenced, then the pest dies. New classes of pesticides, including RNAi-based pesticides, are needed to overcome pesticide resistance and reduce the environmental impacts of pesticides. Unfortunately, dsRNA is easily degraded by enzymes in the environment, particularly those produced by microbes in the soil (Dubelman *et al.,* 2014), severely limiting delivery of dsRNA to cryptic (soil dwelling) species unless transgenic plants are used. Here we investigate dsRNA stability when incubated in corn soil supernatant *ex situ* to determine if encapsulation of dsRNA in chitosan-based nanoparticles (CB-NPs) enhances stability in corn soil. Interestingly, dsRNA stability was not affected by soil supernatant, possibly due to the time of year when sampling was performed (Icoz *et al.,* 2008). Nonetheless, these findings provide insight into dsRNA stability in soil, and in the future may lead to a method for protecting dsRNA from environmental degradation using CB-NPs.

Purpose

Determine if CB-NPs can enhance dsRNA stability in corn soil

Questions, Hypotheses, and Predictions

Question: Can CB-NPs protect dsRNA from being degraded in soil?

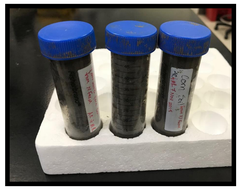
Hypothesis: CB-NPs will defend against degradation of dsRNA

Study System

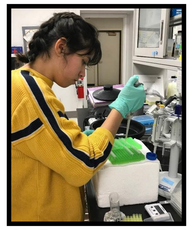
Corn (*Zea mays* L.) is a major grain crop grown in the US, accounting for 95% of the total feed grain annually. Every year more than 90 million acres of land are planted with corn, and used to make livestock feed as well as food and industrial products (Capehart, 2018). Unfortunately, damage from insect pests, such as the western corn rootworm, significantly impact corn yields (Nordhaus, 2017). Due to pesticide resistance and environmental concerns, new tools are needed to combat corn pests. RNAi-based pesticides appear promising; however, dsRNA is completely undetectable in agricultural soil after just two days (Dubelman *et al.,* 2014). Thus strategies for enhancing the environmental stability of dsRNA are needed.

Kansas corn soil

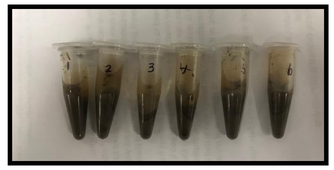
Methods and Experimental Design



1) Collect corn soil samples at KSU N. Farm

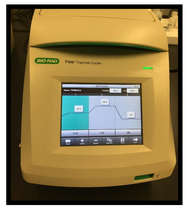


2) Prepare dsRNA & CB-NPs

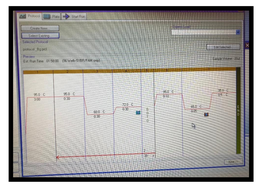


3) Weigh soil & prepare supernatant

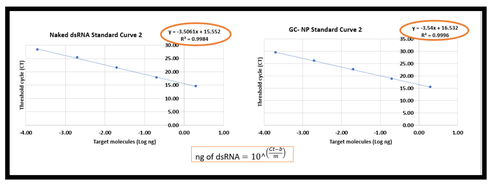
4) Incubate dsRNA & CB-NPs with soil supernatant or PBS



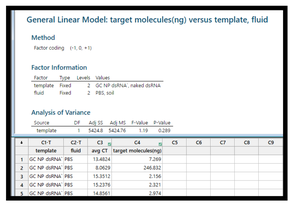
5) Convert dsRNA to cDNA



6) Measure cDNA with RT-qPCR



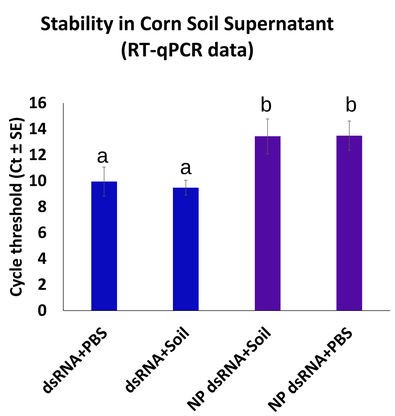
7) Convert Ct values to nanograms of dsRNA based on standard curves

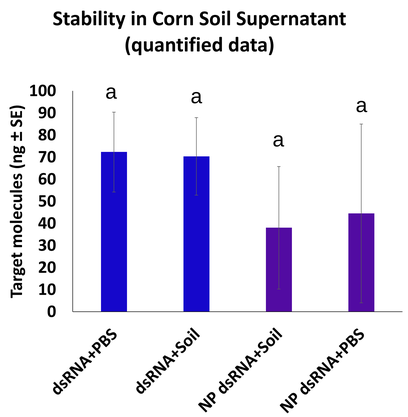


8) Analyze data with a 2-way ANOVA

Results

There was no significant difference between dsRNA incubated in soil supernatant (i.e., dsRNA+Soil) compared to the buffer-only control (i.e., dsRNA+PBS). The failure of this positive control indicates that our soil supernatant did not significantly degrade dsRNA, as occurred during preliminary trials. In addition, the Ct values were significantly later for NP dsRNA (purple bars) than for naked dsRNA (blue bars), indicating that different quantities of target molecules many have been used. For these reasons our data cannot be used to evaluate our hypothesis.





Conclusions

To investigate if CB-NPs can be used to enhance dsRNA stability in corn soil, this study evaluated the ability of CB-NPs to protect dsRNA from degradation in corn soil supernatant. Our findings were inconclusive due to the inability of the soil samples to degrade dsRNA, and further testing is required to properly evaluate our hypothesis.

Future Directions

The soil samples for this study were collected in late fall, whereas optimization trials were conducted during the summer. Other groups report seasonal variations in enzymatic activity of soil microbes (Icoz *et al.,* 2008). Therefore, repeating our experiment with soil collected during the growing season will likely increase the degradation of dsRNA in soil supernatant, allowing us to test our hypothesis. In this manner we hope to examine the true potential for CB-NPs to protect dsRNA in soil during the growing season when target pests are most active. If successful, in the future it may be possible to use CB-NPs to make RNAi-based pesticides more stable in the soil for control of soil-dwelling pests.

When we sampled

When we need to sample

References

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Dubelman, S. J. Fischer, F Zapata, K Huizinga Cj., et al., 2014. Environmental Fate of Double-Stranded RNA in Agricultural Soils. *PLOS* 9(3):e93155.

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