Analysis and production of dsRNA-layered double hydroxides nanocomposites for plant protection

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Layered double hydroxide (LDH) nanoparticles are an increasingly popular tool in biomedical applications as carriers of drugs, chemicals or nucleic acids [1], and have recently been proposed for the control of plant pathogens by RNAi [2]. We are considering LDHs as carriers of viral-derived dsRNAs for the control of virus diseases in vegetable crops, as alternative to traditional breeding or transgenic development. For that, we have developed tools and protocols for *in vitro* and *in vivo* synthesis of specific dsRNAs in addition to the standardized synthesis of LDH nanoparticles. Binding conditions were optimized and the loading capacity of LDHs for dsRNAs was determined. In addition, physical-chemical characterization and microscopy of the particles and nanocomposites was performed by using RAMAN, EDX, IR, Z-potential, PDi, PI, and TEM spectroscopy, among other tools.
dsRNAs (about 500 bp) derived from two genes of each of the cucurbit-infecting viruses *Cucumber green mild mottle virus* (CGMMV) and *Tomato leaf curl New-Delhi virus* (ToLCNDV) have been obtained. LDH nanoparticles were produced by Al-Mg coprecipitation (1: 3). TEM allowed the determination of the particle size, averaging 100 nm, and their structure was found to be laminar hexagonal shapes, characteristic of these nanoparticles. Binding of the dsRNAs to the LDH was performed and the LDH binding ratio of LDH with respect to dsRNA resulted 15: 1 (w/w). dsRNAs seem to attach electrostatically to the laminar LDHs and does not intercalate between sheets.

References

[1] Arrabito G., Bonasera A., Prestopino G. et al., Crystals, 9 (2019).
[2] Fletcher S.J., Reeves P.T., Hoang B.T., Mitter N., Frontiers in Plant Science, 11 (2020).

Figures

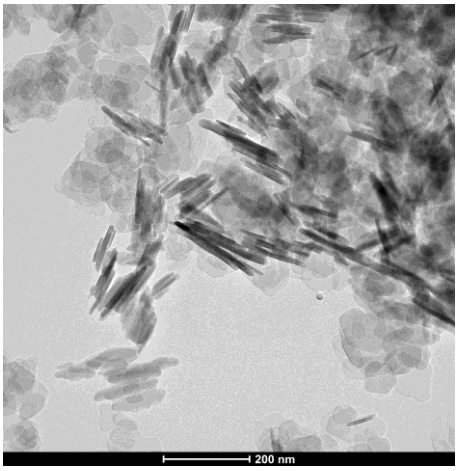

Figure 1: TEM of Mg-Al layered double-hydroxides.

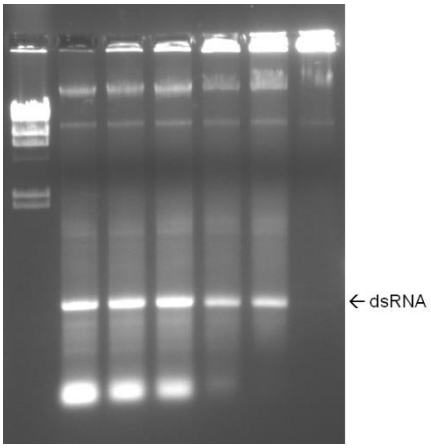

Figure 2: Loading of dsRNA-enriched nucleic acid samples with increasing amounts of LDH in 1% agarose gels stained with RedSafe. LDH-dsRNA nanocomposites do not migrate in electrophoresis.