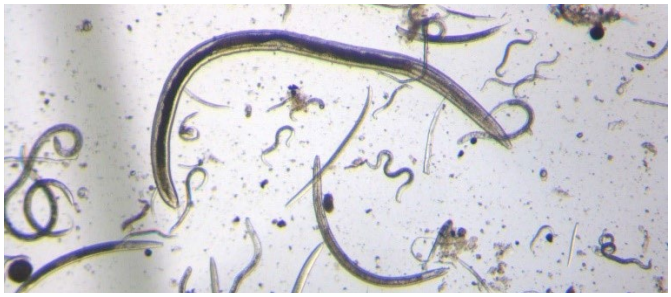
THE WHAT AND WHY

The molecular characterization of soil nematode communities is hampered by the lack of an optimized DNA-extraction method

The characterization of soil nematode communities for (for example) biodiversity assessments is easier, faster and cheaper with DNA-based methods, but for reliable results good quality DNA is a must. Unfortunately, most DNA-extraction methods are optimized to extract DNA from isolated plant-parasitic nematodes or to detect plant-parasitic nematodes in a nematode community. However, all plant-parasitic nematodes together constitute only for about 15% of all nematode species described. In order to be able to obtain DNA from the complete variety of nematodes present in a soil sample, the DNA-extraction methods need to be validated and if needed optimized or newly developed.



1. A diverse nematode community from a soil sample (ILVO).soil sample under the microscope (ILVO).

HOW IS THE CHALLENGE ADDRESSED

An optimized DNA-extraction method aids the characterization of soil nematode communities

The SoildiverAgro-project (H2020 project 817819) developed a new DNA-extraction protocol for a qualitative and quantitative reliable DNA-based soil nematode community characterization. The DNA-extraction protocol combines an enrichment, filtration and bead-beating step with a commercial DNA-extraction kit to optimally extract DNA from nematode communities from diverse soil types and origins. During the enrichment method, nematodes are isolated from the soil by zonal centrifugation. The benefit is that a lot of non-nematode soil organisms are removed that otherwise would interfere with the identification process of the nematodes. Moreover, this enrichment method makes it possible to apply a larger volume of soil sample (100 mL) compared to methods extracting DNA directly from soil (5-10 g). The nematodes in the obtained nematode suspension are collected on a filter (0.8 µM PES membrane filter) by vacuum-filtration. The liquid phase is discarded, the filter with nematodes is transported into a 2mL Eppendorf tube and destroyed by bead-beating with Tungsten-Carbide bullets. The resulting powder is then completely used for DNA-extraction. Previous steps thus concentrate the nematode suspension to make the volume suitable for commercial DNA-extraction kits and also destroy all nematodes for a qualitative and quantitative nematode community characterization. The DNeasy Powerlyzer Powersoil DNA-extraction kit (Qiagen), containing a chemical lysis step, was implemented into the protocol. This kit further destroys nematode cells and adds several purification steps to further remove soil chemical substances. The result is pure, good quality DNA. Tests revealed that our DNA-extraction protocol is more efficient and sensitive than previous protocols.

2. AZC, automated zonal centrifuge, is a specialized machine able to isolate approximately 95% of all nematodes from a soil sample (100mL) into a small beaker (40mL nematode suspension).

3. Vacuum-filtration apparatus with (from top till bottom) a suspension reservoir, a clamp, a filter holder, a rubber seal and an Erlenmeyer flask with outlet.

KEYWORDS

DNA-extraction, molecular characterization, nematode communities, soil, vacuum-filtration, zonal centrifugation.

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