An effective approach to retrieve cultivable Acinetobacter spp. from the soil environment

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AIM
To design and test an effective approach to recover taxonomically diverse Acinetobacter strains from environmental soil samples.

INTRODUCTION
A basic prerequisite for understanding the role of a given group of bacteria in ecological processes is a comprehensive knowledge of their physiological and metabolic features at the species level. To gather such information, obtaining pure bacterial cultures and their laboratory characterization are inevitable.

Bacteria of the ubiquitous genus Acinetobacter play an important role in biological processes in soil ecosystems. However, the taxonomic diversity of these bacteria in natural environments is largely unknown.

RESULTS
Sample A yielded 11 unique Acinetobacter strains. Of these, four were allocated to three known species while seven represented novel species (Fig. 2).

Sample B showed an extremely high species diversity: 16 strains were classified as seven known and five new species (Fig. 2).

Sample C produced 10 strains belonging to three known and three novel species (Fig. 2).

METHODS
Samples were processed as shown in Fig. 1. MALDI-TOF MS identification was performed by matching the obtained spectra to those of the current Bruker Daltonics database supplemented with homemade entries representing all known and provisional Acinetobacter spp.

STRAINS
The final classification of strains at the species level was based on the comparative analysis of the rpoB gene, MALDI-TOF MS and comprehensive physiological and metabolic testing (Fig. 2).

Fig. 1. A stepwise approach for a soil sample treatment and for the identification of Acinetobacter isolates.

Fig. 2. Rooted neighbour-joining tree based on the partial nucleotide sequence of the rpoB gene (861 nt) of environmental strains of the samples A, B, C and the representatives of all known Acinetobacter spp. (in black). Bootstrap percentages (≥75%) after 1000 simulations are shown. The sequence of Pseudomonas aeruginosa PA01 (NCBI accession no. NC002516) was used as the outgroup. Bar, 0.05 substitutions per nucleotide site.

CONCLUSION
We have developed an effective approach which allows for quick taxonomic screening of hundreds of colonies and enables effective recovery of taxonomically diverse Acinetobacter strains from environmental samples.