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

Lenka Křížová & Alexandr Nemec

Laboratory of Bacterial Genetics, National Institute of Public Health, Prague, Czech Republic

AIM

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



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

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.





Samples A, B and **C**, collected during 2014, originated from a tropical rainforest in Sumatra (Indonesia) and two protected wetland areas in the Czech Republic, respectively.



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**).



Acinetobacter radioresistens NIPH 513^T Acinetobacter qingfengensis ANC 4671[⊤] Acinetobacter puyangensis ANC 4466^T Acinetobacter soli NIPH 3874[⊤] Acinetobacter baylyi NIPH 2312 Acinetobacter ursingii NIPH 137^T Acinetobacter apis ANC 5114[⊤] Acinetobacter nectaris ANC 4385^T Acinetobacter boissieri ANC 4422^T Acinetobacter junii NIPH 511^T Acinetobacter junii ANC 5051 Acinetobacter junii ANC 4948 Genomic sp. 6 NIPH 1853 Acinetobacter haemolyticus NIPH 510^T Acinetobacter tandoii NIPH 2284^T Acinetobacter towneri-like ANC 5045 Acinetobacter towneri ANC 4865 Acinetobacter towneri NIPH 2286^T Ungrouped ANC 4942 Acinetobacter brisouii ANC 4119[⊤] Acinetobacter rudis NIPH 4129[⊤] Acinetobacter gerneri NIPH 2282[⊤]

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.¹

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. 2. Rooted neighbour-joining tree based on the

Acinetobacter guillouiae NIPH 522^T Acinetobacter bereziniae NIPH 521¹ Acinetobacter bereziniae ANC 4950 Ungrouped ANC 5054 Ungrouped ANC 4945 Acinetobacter bouvetii ANC 5042 Acinetobacter bouvetii NIPH 2281[⊤] Acinetobacter bouvetii-like A ANC 5057 Ungrouped ANC 4946 Taxon 23 ANC 5044 Acinetobacter Iwoffii NIPH 512^T Acinetobacter Iwoffii ANC 5055 Acinetobacter variabilis NIPH 2171[⊤] Acinetobacter indicus ANC 4215^T Acinetobacter guangdongensis ANC 5077 Acinetobacter schindleri ANC 5049 Acinetobacter schindleri NIPH 1034[⊤] Acinetobacter kookii ANC 5048 Acinetobacter kookii ANC 5050 Acinetobacter kookii ANC 4667^T Taxon 32 ANC 5043 Taxon 32 ANC 5046 Taxon 31 ANC 4874 Acinetobacter gandensis ANC 4864 Acinetobacter gandensis ANC 4275[⊤] Acinetobacter bohemicus ANC 3994^T Acinetobacter bohemicus ANC 4867 Acinetobacter bohemicus ANC 5053 Acinetobacter bohemicus ANC 4866 Acinetobacter bohemicus ANC 4868 Acinetobacter harbinensis ANC 4817[™] Ungrouped ANC 5047 Ungrouped ANC 4862 Acinetobacter johnsonii ANC 4870 Acinetobacter johnsonii NIPH 518[⊤] Acinetobacter johnsonii ANC 4871 Acinetobacter johnsonii ANC 5041 Acinetobacter johnsonii ANC 4869 Acinetobacter baumannii ANC 5052 Acinetobacter baumannii NIPH 501^T Acinetobacter seifertii NIPH 973^T Acinetobacter nosocomialis NIPH 2119^T

Acinetobacter nosocomialis ANC 4947 Acinetobacter pittii ANC 4949 Acinetobacter pittii NIPH 519^T Acinetobacter calcoaceticus NIPH 2245[™] Acinetobacter calcoaceticus-like ANC 4941 Acinetobacter calcoaceticus-like NIPH 542 Acinetobacter beijerinckii NIPH 838^T Acinetobacter venetianus NIPH 1925^T Genomic sp. 13BJ NIPH 1860 Genomic sp. 14BJ NIPH 1847 Acinetobacter parvus NIPH 384[⊤] Ungrouped ANC 4944 Genomic sp. 16 NIPH 1872 Genomic sp. 15BJ NIPH 1866

Acinetobacter gyllenbergii NIPH 2150^T

Acinetobacter tjernbergiae NIPH 2285^T

Ungrouped ANC 4943

Ungrouped ANC 4940

Genomic sp. 17 NIPH 1867







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 PAO1 (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.

Presented as Poster P SOIL 56 at the 13th Symposium on Bacterial Genetics and Ecology, 14 – 18 June 2015, Milan, Italy. Funding: Project no. 13-26693S of the Czech Science Foundation. References: ¹Touchon et al. 2014; Genome Biol Evol 6: 2866-2882. **Correspondence:** krizova.work@gmail.com.