Widespread distribution of *Rhizobium radiobacter* in Mediterranean sediments

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**Introduction**

In sediments of the eastern Mediterranean Sea, dark sediment layers, called sapropels, occur periodically every 20,000 years. They are highly enriched in organic carbon and were formed in response to increased nutrient input, productivity and anoxic deep water formation. Sapropels contain higher cell numbers and activities than carbon-lean intermediate layers demonstrating the presence of active microbial communities [1]. In a cultivation approach, performed during Meteor cruise Leg 51/3 in November 2001, up to 3.3 % of the microbial community of sapropels were enriched and 175 pure cultures were obtained [2]. A molecular screening revealed that 20 % of all strains were closely related to the soil bacterium *Rhizobium radiobacter*. The question arisen whether this bacterium might be an indigenous microbe of sapropels.

**Methods**

A quantitative PCR assay with SybrGreenI as fluorescent dye was developed for the quantification of *Rhizobium radiobacter* in environmental DNA extracts. A *Rhizobium* sp. specific primer was designed and combined with a specific primer for α-Proteobacteria. Specificity studies were performed with 38 non-target strains from the DSMZ and from sapropels. As standards for PCR quantification, purified 16S rDNA fragments of the environmental strain J117 were prepared. A set of experiments was performed to test the effect of background DNA and the accuracy over a broad range of quantifications. Measurements of samples containing defined amounts of *Rhizobium* sp. and E. coli DNA revealed a sufficient accuracy over a broad range of specific target proportions. E. coli background DNA had no effect on quantifications.

**Results**

The quantitative PCR assay was linear over six orders of magnitude and allowed the highly specific detection of as little as 10 *Rhizobium* sp. targets in environmental samples.

**Conclusions**

The developed qPCR protocol allowed a highly specific, sensitive and accurate quantification of *Rhizobium radiobacter*.

*R. radiobacter* is widespread in sediments of the Eastern Mediterranean, contributing a substantial part of the active microbial community in sapropel layers.

This is the first study in which the *in situ* abundance of a cultivated deep biosphere bacterium was demonstrated by molecular techniques.

**Literature**

