

# Characterization and evaluation of tandem repeats for the identification of *Geobacillus*

Research Article

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**Abstract:** Taxonomy of thermophilic, endospore-forming bacteria has evoked a great interest over the past few years. Although a number of taxonomic markers were previously evaluated, their sequences in *Geobacillus* were too conservative, and identification of more variable markers is needed. Repetitive DNA is one of the promising variable targets in the development of the taxon-specific genotyping and identification schemes in bacteria. The aim of our study was to evaluate the possibility of using repetitive DNA in the taxonomy of *Geobacillus*. In this paper, we report the analysis of perfect tandem repeats of geobacilli. We focused on the long repeats (with a motif length of  $\geq 20$  nucleotides). This choice was based on the assumption that these motifs can be used for the construction of oligonucleotides - primers and probes. Thirty-three *Geobacillus* genus-specific motifs were identified in our work, fifteen of them were species-specific and fifteen - species cluster -specific. Three of them were genus-, but not species- or species cluster-specific. Some of the motifs were used for the construction of the primer pairs. The primers were validated by PCR. Out of 12 designed primer pairs, 11 were genus-specific and 4 - species-specific. Species-specific primers were successfully constructed for the phylogenetically defined species *Geobacillus thermodenitrificans* and *Geobacillus toebii*.

**Keywords:** *Geobacillus* • Tandem repeat • Repetitive DNA • Long repeat • Genus-specific • Species-specific

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## 1. Introduction

Taxonomy of thermophilic, endospore-forming bacteria has evoked a great interest over the past few years [1-5]. Thermophilic endospore-formers constitute an excellent resource of biotechnologically important products and can be used as the producers of these proteins [6-10]. It is essential to monitor the purity of the protein-producing strains in these processes. Thermophilic, endospore-forming bacteria are also associated with heat-treated foods; spores of thermophilic *Geobacillus* species are a common contaminant of milk powder worldwide [11]. Hence, the monitoring and characterization of thermophilic microbial contamination are very important for both the food industry and biotechnology. In order to simply and effectively monitor the presence of certain species, or even strains of geobacilli, rapid, convenient

and reliable methods of identification and genotyping are needed. A number of taxonomic markers (*rpoB* [12], *recA* [13], *recN* [14], *spo0A* [15]) were previously evaluated, but their sequences in *Geobacillus* were too conservative, and identification of more variable markers is needed.

Repetitive DNA is one of the promising variable targets in the development of the taxon-specific genotyping schemes in bacteria [16]. Previous studies have shown that primers targeting repeated sequences enhance the sensitivity of DNA detection in comparison with regular PCR [17-19]. Consequently, fewer copies of the DNA can be detected in the sample when using repeats-targeted PCR.

The aim of our study was to evaluate the possibility of using repetitive DNA for the taxonomy of *Geobacillus*. In this paper we report the analysis of tandem repeats

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