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Constructing robust and efficient PCR-based systems to discriminate *Bacillus cereus* subspecies

Antonets K.S.[1, 2]*, Malovichko Y.V.[1, 2], Merkushova A.[1, 3], Shikov A.E.[1, 2], Nizhnikov A.A.[1, 2]

[1] *All-Russia Research Institute for Agricultural Microbiology (ARRIAM), Pushkin, St. Petersburg, Russia*

[2] *St. Petersburg State University, St. Petersburg, Russia*

[3] *Saint Petersburg National Research Academic University, St. Petersburg, Russia*

** k.antonets@arriam.ru*

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Motivation and Aim: The bacterium *Bacillus thuringiensis*, actively used as biocontrol agent against insect pests, is a part of *Bacillus cereus* group. This group now includes a great variety of species from agriculturaly important to severely pathogenic among them with unclear systematics. Due to rapid divergence and active horizontal gene transfer it is practicaly impossible to find "universal" marker to discriminate one species from another. In this work we tried to construct the minimal set of markers which allows to classify strains within *B. cereus* group using PCR.

Methods and Algorithms: We constructed the initial dataset using genome assemblies attributed to species and subspecies withing *B. cereus* group and available at the NCBI database. The marker set has been constructed by slicing the assemblies into 1 kb fragments and constructing presence/absence table for them.

Results: We have developed a workflow that is able to construct a set of markers for classifying bacteria with a certain group based on the representative genomes. The developed workflow has been used to predict such markers for the bacteria in *B. cereus* group. The ability of this set of markers to classify strains withing *B. cereus* group has been validated *in silico* and experimentally using qPCR.

Conclusion: In spite of the high complexity of genome evolution in *B. cereus* group our approach has been able to find a combination of PCR markers for classifying strains within this group. Using this set of markers for the first time allows high-throughput classification of new strains without expensive whole genome sequencing.

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