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**Filogeneza i zmienność genomowa przedstawicieli
Bacillus cereus sensu stricto z gleb Eurazji,
Afryki i Ameryki Południowej**

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ABSTRACT

Bacillus cereus sensu stricto (*B. cereus*) are Gram-positive, aerobic and spore-forming bacteria belonging to *Bacillus cereus sensu lato* (*B. cereus* group). Members of the group display a high degree of genetic similarity, but different phenotypic features, mainly transferred on plasmids, will allow them to be classified into separate species. The highest number of *B. cereus s.l.* isolates are classified as *B. cereus*, bacilli widely distributed in various environments. These bacilli sporadically cause food poisoning and intestinal infections. *B. anthracis*, another member of the group, is a very important in the area of human economy and health as a factor causing anthrax – dangerous disease of human and ruminants. In turn, *B. thuringiensis* producing insecticidal proteins, is used in biopesticides. The main reservoir of *B. cereus s.l.* is the soil, from endospores are transmitted to foodstuffs, dust or animal digestive tracts. Until now the occurrence of the gene transfer process (for example virulence genes) between members of the *B. cereus* group has been repeatedly revealed.

The aims of this PhD thesis were determine (i) the degree of genetic polymorphism, (ii) genetic populations structures, (iii) reconstruction of phylogenetic relationships and (iv) determination of genome variability of *B. cereus* isolates from soil samples collected in Argentina, Kazakhstan, Kenya and Poland. These analysis made it possible to get acquainted with the properties of the above bacteria in the aspect of the specific geographical location and in the intercontinental dimension.

The application of typing based on nucleotide fragments of several loci (MLST) allowed to obtain molecular data for the study of genetic differentiation and phylogeny of *B. cereus* isolates. Analyzes of nucleotide sequence by Ecotype Simulation algorithm made it possible to designate putative ecotypes among the studied populations. Additionally, on the basis of the presence of genetic determinants of the RepA and RepX replication proteins, the frequency of the pXO1- and pXO2-like plasmids of *B. anthracis* among soil *B. cereus* isolates were estimated. In turn, the sequencing of replicons of pXO2 plasmids enabled the comparison of their organization and nucleotide structures.

Analyzes aimed at estimating genetic polymorphism among four geographically distinct soil populations of *B. cereus* showed a high degree of differentiation in all of them (identification of 354 alleles), while the Polish population revealed the highest level of diversity (385 polymorphic sites among 40 sequence types). The number of assigned STs in individual locations was differentiated, with all Kenyan isolates classified into separate, previously unwritten sequence types (42 STs). Comparative analysis showed similar evolutionary tendencies in all examined

loci in the form of the number of polymorphic sites, regardless of the origin. However, the determined ranges of r/m coefficients indicate a variable frequency of recombination occurrence in individual populations (from 0,70 among the isolates from Argentina to 3,70 among the isolates from Kenya and Poland). On the other hand, low values of dN/dS factors (from 0,027 to 0,038) suggesting the clonality of all populations was not reflected in their genetic structures, because only 37% isolates from 161 *B. cereus* form clonal complexes.

The reconstruction of phylogeny in individual populations revealed a different degree of relationship with the *B. cereus sensu lato* reference strains. Bacilli from Argentina and Kenya did not show phylogenetic relationships with psychrotolerant strains, unlike to *B. cereus* from Kazakhstan, which suggests the ecotypic nature of the *B. cereus* populations, probably associated with different climatic conditions. Establishing phylogenetic relationships in the intercontinental aspects showed a tendency to form five separate clades, corresponding to the origin of the isolates. Importantly, none of them created bacteria from only one continent.

The ubiquity of the carriage of pXO1- and pXO2-like plasmids among soil *B. cereus* has been demonstrated in all studied populations. The frequency of pXO2-like positive isolates in all cases exceeding the number of pXO1-like positive bacilli. The relative degree of expression of the genes encoding the replication proteins of these plasmids at the transcriptome level has been proved more homogenous in all populations in the case of the *repA_N* gene, carried on pXO2-like plasmids. Comparative analyzes with the replicon of *B. anthracis* pXO2 plasmid revealed the same arrangement of its elements. However, the nucleotide sequences of *B. cereus* replicons revealed the presence of characteristic nucleotide substitutions among soil isolates, distinguishing their replicons from the *B. anthracis*.

The study presented in the doctoral thesis expand our knowledge on the genetic nature of environmental *B. cereus* populations.

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