DAMAGE-INDUCED MUTATION CLUSTERS IN *B. SUBTILIS*

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Introduction: Multiple studies on different biological systems showed the presence of the non-random distribution of mutations in the genome. Particularly, mutations are located in a limited space of the genome forming a cluster or hotspots, while the remaining genomic area remains virtually intact. Whole-genome sequencing of chemically mutagenised gram-negative *E.coli* showed a strikingly non-uniform pattern of mutations distribution, which was arranged in clusters in several sequenced *E.coli* genomes. We used this strategy to extend study in gram-negative bacteria to gram-positive ones to determine whether this phenomenon is more universal and not confined only to *E.coli.* Use of next-generation sequencing approach and bioinformatic tools allowed analyzing several mutagenised *B.subtilis* genomes.

Methods: Mutagenesis in gram-positive *Bacillus subtilis subsp. subtilis str. 168* using alkylating agent ethyl methanesulfonate was induced. Five randomly picked single colonies were taken for the analysis of mutation distribution through Ion Torrent PGM sequencing platform. Data analysis was conducted through bioinformatic pipeline using programs and tools as Cutadapt, Samtools, BWA, VarScan and IGV.

Results: Whole-genome sequencing of several mutagenized *B.subtilis* genomes through Ion Torrent PGM showed a non-random dispersion of mutations caused by the EMS treatment. Variant calling showed high number of G-to-A and C-to-T transitions that were asymmetrically distributed throughout the genomes in the form of mutation clusters of different length. The "stretch"of one mutation type was switched to the "stretch" of another mutation type. The pattern of such "stretches" and "switches" of C-to-T and G-to-A coordinated mutations was similar to the pattern observed in the work on gram-negative *E.coli.*

Conclusion: In the current study the use of a whole-genome sequencing approach allowed to analyze several EMS-mutagenised *B.subtilis* genomes. Observed asymmetrical mutation pattern in the EMS-treated gram-positive *B.subtilis* indicates not only the universality of the phenomenon of mutation clusters, but also the effectiveness of next-generation sequencing in detecting non-uniform mutation distribution pattern on the genomic scale.