doi 10.18699/bgrs2024-5.2-25

Evolution and distribution of prokaryotic type III toxin-antitoxin systems

Selifonov I.[1, 2]*, Moshenskiy D.[2], Alekseevskiy A.[1, 2, 3]

[1] *Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Russia*

[2] *Department of mathematical methods in biology, A.N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia*

[3] *Research Institute for System Analysis, RAS, Moscow, Russia*

** selifonov2002@gmail.com*

Key words: bacteria; defense systems; toxin-antitoxin systems

Motivation and Aim: A prokaryotic toxin-antitoxin (TA) system consists of two main components: a toxin that inhibits growth or triggers programmed cell death and a corresponding less stable antitoxin that neutralizes the former. Though these systems can be considered as selfish genetic elements that enforce their own stable vertical inheritance, for many of them a wide variety of functions have been proposed [1]. Type III TA systems consist of a protein toxin and a small ncRNA antitoxin, which is encoded in a series of repeats directly preceding the toxin gene. The latest thorough bioinformatic analysis of the evolution and distribution of these systems was performed by Blower et al. in 2012, when they were separated into three families: toxIN, tenpIN and cptIN based on the sequence similarity of the toxins [2]. In this work we revisit this topic with new genomic data. The aim of this work is to assess the diversity and phylogenetic distribution of prokaryotic type III TA systems.

Methods and Algorithms: Using the 125 classified toxin sequences, we built multiple sequence alignments for each of the 3 families. After manual curation and trimming of the alignments they were used to create 3 HMM profiles. Using the profiles, we performed searches in the original protein sequences, which let us define the bit score gathering thresholds for the 3 toxin groups. For each protein family the value of the threshold was set to the score of the weakest (the least statistically significant) correct hit of this family's profile. Though the real score distribution of type III toxins is likely not as narrow as the one observed in the sample of 125 proteins, the use of such strict thresholds helped reduce the amount of noise in the data, which could otherwise complicate its further analysis.

The HMM profiles were then used to search for toxin homologs in 40359 prokaryotic genomes downloaded from the NCBI database. The resulting protein sequences with domain scores equal to or higher than the thresholds were included in the corresponding families. In order to identify the antitoxin DNA repeats we searched for sequence motifs in the fragments including 800 bases upstream of the coding sequence of each toxin gene.

To characterize the evolutionary dynamics of type III TA systems phylogenetic trees were built for unique toxin sequences and compared with reference trees created for the same bacteria based on the protein sequences for 107 essential single-copy core genes.

Results: By the described approach 610 putative toxins were found and classified (Table 1). For 484 of them we were also able to identify their cognate antitoxins.

Table 1. The number of found toxins for each family and the results of searching for antitoxin repeats

Family	Total systems	Number of repeats	Repeat length	No repeats found
toxIN	342	3.29 ± 2.07	33	33
cptIN	119	2.76 ± 1.90	39	37
tenpIN	149	2.37 ± 2.19	39	56

The newly described type III TA systems displayed an uneven phylogenetic distribution. Moreover, different taxa were enriched with toxins belonging to different families (Fig. 1).

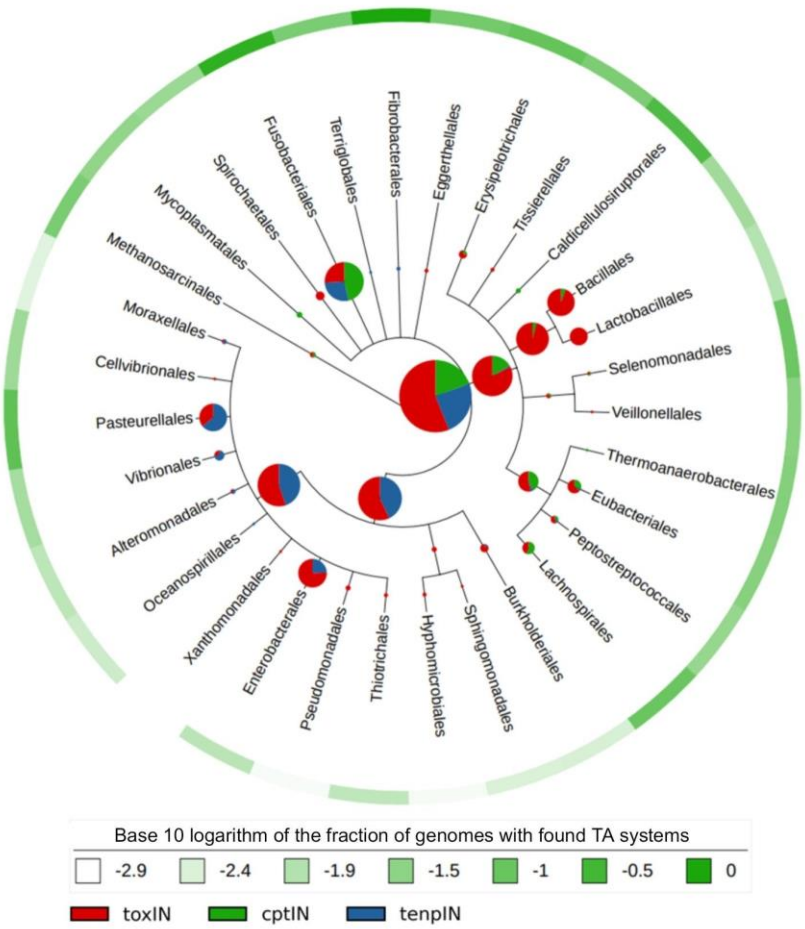

Fig. 1. The taxonomic distribution of type III TA systems of different families

The comparison of the phylogenetic trees built for unique toxin sequences and the corresponding strains of bacteria showed that TA loci frequently undergo horizontal gene transfer between different species and genera of microorganisms.

Conclusion: The 3 TA families differ in the sequence and organization of antitoxin repeats and are usually found in different taxa. Horizontal gene transfer between both closely related and distant groups of prokaryotes plays a significant role in the evolution and spread of type III TA systems.

Funding: The study is supported by the grant RSF No. 21-14-00135.

References

1. Jurėnas D., Fraikin N., Goormaghtigh F., van Melderen L. Biology and evolution of bacterial toxin–antitoxin systems. *Nat Rev Microbiol*. 2022;20(6):335-350. doi 10.1038/s41579-021-00661-1
2. Blower T.R., Short F.L., Rao F., Mizuguchi K., Pei X.Y., Fineran P.C., Luisi B.F., Salmond G.P.C. Identification and classification of bacterial Type III toxin–antitoxin systems encoded in chromosomal and plasmid genomes. *Nucleic Acids Res*. 2012;40(13):6158-6173. doi 10.1093/nar/gks231