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Phylogenetic interrelations between serological variants of *Bacillus thuringiensis*

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Aim. *B. thuringiensis (Bt) are gram-positive spore-forming aerobic or facultative anaerobic bacteria able to form during sporulation species specific crystal-like inclusions of protein nature, consisting of particular thermolabile -endotoxins. Serological Bt variants produce different entomotoxins; their synthesis in many respects depends on the conditions of cultivation. There was accumulated a vast information on the entomotoxins, their origin, synthesis, structure, toxic properties and mechanisms of action on insects. These bacteria are dominating in the microbiomethods of pest control in plants and animals. There are more than 70 serovariants of Bt selectively specific to the definite groups of host insects. However, the description of new variants not always looks justified considering the phylogenetic systematization based on phenotype signs.* ***Methods***. *A comparative phylogenetic analysis of the Bt intraspecific interrelations was performed on the basis of the cloned 16S rRNA genes of entomopathogenic bacteria* BtH_1, BtH_{10}, BtH_{14}. ***Results***. *The phylogenetically homogeneous lines were investigated – a homology of 16S rRNA of the strains 1 and 10 ranged from 90,0 to 94,0 %; no distinct genetic isolation among the strains of 14th and 10th serovars was revealed.* ***Conclusions***. *The comparison of nucleotides sequences of 16S rRNA has shown the existence of strains polymorphism within the group of entomopathogens* BtH_1, BtH_{10}, BtH_{14}, *connected with their entomocide activity.*

Keywords: serovars, Bacillus thuringiensis, phylogenetic analysis.

Introduction. The use of genome or its fragments in the systematization of microorganisms is of special importance due to its relative conservatism compared to any other features; besides, genetic differences better reflect evolutionary formed relations between microorganisms, which makes them a good foundation for modern classification of bacteria.

The application of the methods of evaluating genetic similarity (comparative DNA analysis and analysis of amino acid sequences of proteins) allows extending the possibilities of researchers in creating molecular evolutionary systematics and phylogeny of bacteria. The phylogeny of a group of organisms is traditionally presented in the form of hierarchic “tree”, reflecting possible evolutionary relations [1, 2]. The application of molecular and genetic approaches in

The list of Bacillus thuringiensis strains, used in the study

Variant	Serovariant	Number of strains	Abbreviated name (according to the catalogue of Institute Pasteur)	Functional group
thuringiensis	1	5	THU	I
darmstadiensis	10a, 10b	4	DAR	IV
israelensis	14	3	ISR	IV

microbiology resulted in the establishment of several directions in the systematization of bacteria, in particular, the formation of phylogenetic systematization of bacteria, identification of strains using phylogenetic and phenotypic information, and detection of microorganisms in the environment without their cultivation. It allows controlling the purity of biopreparations, based on active, technological strains, and monitoring cultures in corresponding conditions. There is a need for foundation of further studying on genotype of cultures, promising for biotechnologies, as well as for the search and detection of genes, predetermining high biological activity.

B. thuringiensis (Bt) are gram-positive spore-forming aerobic or facultative anaerobic bacteria able to form species specific crystal-like inclusions of protein nature, consisting of particular thermolabile -endotoxins, during sporulation. Serological *Bt* variants produce different entomotoxins; in many respects their synthesis depends on the conditions of cultivation. There was accumulated a vast information on the entomotoxins, their origin, synthesis, structure, toxic properties, and mechanisms of their action on insects.

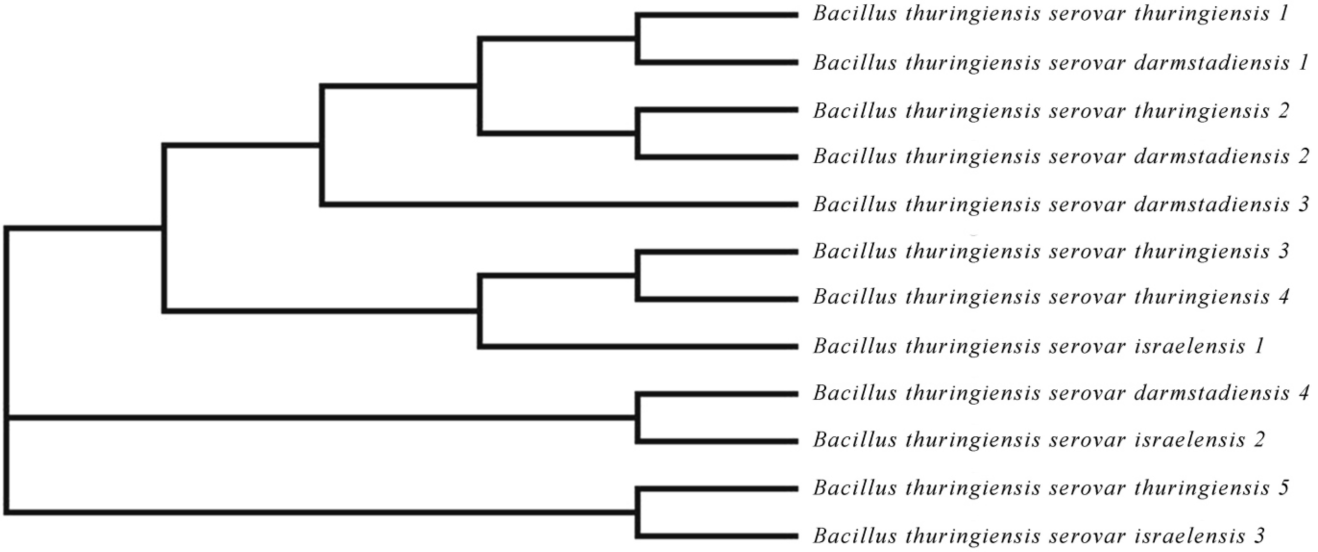
Bt strains are pathogenic for larvae of Colorado potato beetle, younger larvae of Mamestra brassicae, *Pieris brassicae*, *Plutella maculipennis*, *Phyllotreta atra F.* and *Brevicoryne brassicae* on vegetables. They are highly remarkable for their activity against populations of *Yaponomeuta padellus L.*, *Hyphantria cunea Drury*, *Archips crataegana Hb.*, *Malacosoma neustria L., Ocneria dispar L.*, *Dendrolimus pini L., D. sibiricus*, *Operophtera brumata Cl., Erannis difoliaria L., Euproctis chrysorrhoea, E. karghalica M.* on fruit trees; Tetranychus urticae *Koch., T. telarius L.* on cucumbers in protected soil; caterpillar of *Loxostege sticticalis L.* on beets, carrots, cabbage, sunflower and permanent grasses; caterpillar of *Tortrix viridana L.* on vineyards and many other pests [3].

Therefore, *Bt* are dominating in the microbiomethods of pest control in plants and animals. There are more than 70 serovariants of *Bt*, selectively specific to the definite groups of host insects. However, the description of new variants does not always seem justified considering the phylogenetic systematization based on phenotype features.

The current work is aimed at phylogenetic analysis of various serological variants of entomopathogenic *Bt* bacteria, isolated from natural populations of insects, which is based on the study on polymorphism of nucleotide sequences of 16S rRNA genes.

Materials and Methods. The different strains of entomopathogenic *Bt* bacteria, isolated from natural populations of insects, were used in the work, namely, exotoxinogenic *Bt* var. *thuringiensis* (BtH_{1}), *Bt* var. *darmstadiensis* (BtH_{10}), *Bt* var. *israelensis* (BtH_{14}) (Table).

The DNA of entomopathogenic bacteria was extracted using the method, described in [4]. After electrophoretic separation in 1% agarose gel the samples of DNA obtained were visually detected as described in [5]. Polymerase chain reaction (PCR) for 16S rRNA was performed according to the standard procedure in the amplifier BioRad My Cycler, using oligonucleotides SSU-642-F HAATHYGTGCCAGCAGC and SSU-1445-R GTCRTCCYDCCTTCCTC. After extraction from 1% agarose gel the products of amplification were used for cloning in vector *pAL-TA* [6, 7].



Phylogenetic interrelation of *Bt* variants, based on the analysis of sequences of 16S rRNA genes

Automatic sequencer CEQ 8000 Genetic Analysis System (*Beckman Coulter*, USA) was used to analyse nucleotide sequences and to check their identity to corresponding sequences of 16S rRNAs of various *Bt* variants from the GenBank database. Obtained nucleotide sequences were used to build the dendrogram and to estimate the similarity of serological variants by Vector NTI Advance 8.0 software.

Results and Discussion. The phylogenetic analysis is based on the comparison of genes or proteins, similar in structure or functions, starting with their primary sequences. The analysis of 16S rRNA proved to be a more efficient molecular and taxonomic means in evaluating genetic variety and relations between bacterial kinds (serotypes) of *Bt* entomopathogens, compared to approaches, based on phenotypic data (e.g. serotyping by *H*-antigen). However, it is noteworthy that reconstructions of phylogenetic relations between strains, conducted on the basis of different molecular data are not always in good agreement with "morphological" classification or among themselves. The main reasons of divergence of morphological and molecular reconstructions are based on the fact that only a small part of genetic information, enclosed in the genome of the organism, is revealed on the morphological level. The combination of data of the study on 16S rRNA may be used to obtain visually evident fingerprints and to confirm species membership of strains and phylogenetic interrelations inside the specific species.

The dendrogram, built on the basis of data obtained (Figure), demonstrates existing interrelations between *Bt* strains of the 1st, 10th, and 14th serotypes. Almost all *Bt* types may be divided into groups; they demonstrate phylogenetic similarity, however, there are some evident isolated variants. The topology of dividing bacilli types on the dendrogram testifies to the existence of three main clusters, corresponding to six genotypes. The comparison of *Bt* strains showed that genetically-wise there are three closely-related genotypes of the first cluster with the similarity level of 90.0–93.5% (BtH_1 *(thuringiensis*), demonstrating entomocide activity regarding *Lepidoptera*, and BtH_{10} *(darmstadiensis)*, active against *Coleoptera*. Thus, genetic homology of entomopathogenic strains is also confirmed by the spectrum of their entomocide activity.

The BtH_{14} *(israelensis)* strains, affecting the larvea of *Culicidae*, gnat and herbovorous mosquitoes

(*Cricotopus silvestris, Licoriella fucorum*, etc.) are in the second and the third clusters with the smallest similarity level of 16S rRNA (less than 70% homology). It is noteworthy that the unique effect of *israelensis* bacteria on the larvae of dipterans, including larvicidal effect, is solely related to the specificities of their crystalline endotoxin.

Bt strains, producing various sets of entomotoxins, may vary greatly in their biological activity regarding susceptible insects or show considerable similarities, e.g. some mosquitocide strains, etc. The entomopathogens of the same type are known for their frequently observed similar set of pathogenicity factors as well as for similar biochemical and genome characteristics. However, the descendants of different clones of the same type are specific for some variability both on genotype and phenotype levels.

Therefore, the molecular and genetic analysis of BtH_1, BtH_{10}, BtH_{14}, based on the comparison of nucleotide sequences of 16S rRNA, revealed existence of strain polymorphism within the group of entomopathogens. The differences, defined by morphological, physiological and biochemical, and serological characteristics of *Bt* strains, allow making an interim conclusion on their belonging to at least two phylogenetically homogeneous lines. The similarity level of 16S rRNA of strains of the 1st and 10th serotypes is 90.0–94.0%. There was no distinct phylogenetic isolation revealed between the strains of the 10th and 14th serotypes. The reliability of phylogenetic results will depend greatly on the combination of various data which will give a possibility in future to influence the congruence of phylogenetic trees, statistical support, and definition of their branches, eliminating the systematic error.

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Филогенетические взаимосвязи серологических вариантов *Bacillus thuringiensis*

Цель. *B. thuringiensis (Bt) – грамположительные спорообразующие аэробные или факультативно анаэробные бактерии, способные в процессе споруляции образовывать видоспецифические кристаллообразные включения белковой природы, состоящие из особых термолабильных -эндотоксинов. Серологические варианты Bt продуцируют разные энтомотоксины, их синтез во многом зависит от условий культивирования культуры. Накоплен богатый фактический материал о происхождении энтомотоксинов, условиях синтеза, строении, составе, токсических свойствах и механизмах действия на насекомых. Bt приобрели доминантное положение в микробиометоде борьбы с вредителями растений и животных. В настоящее время существуют разновидности Bt в более чем 70 вариантах (серотипах), избирательно специфичных к определенному кругу хозяев-насекомых. Однако описание новых разновидностей не всегда выглядит оправданным с точки зрения филогенетической систематики, основанной на фенотипических признаках.* ***Методы***. *На основе клонированных генов 16S рРНК энтомопатогенных бактерий BtH1, BtH10, BtH14 проведен сравнительный филогенетический анализ внутривидовых взаимосвязей Bt.* ***Результаты***. *Прослежены филогенетически однородные линии (уровень сходства 16S рРНК штаммов 1-го и 10-го серотипов составляет от 90,0 до 94%; отчетливой генетической обособленности среди штаммов 10-го и 14-го серотипов не выявлено).* ***Выводы***. *При сравнении нуклеотидных последовательностей по 16S рРНК установлено существование штаммового полиморфизма внутри группы энтомопатогенов BtH1, BtH10, BtH14, связанного с их энтомоцидной активностью.*

Ключевые слова: сероварианты, Bacillus thuringiensis, филогенетический анализ.

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Філогенетичні взаємозв'язки серологічних варіантів *Bacillus thuringiensis*

Резюме

Мета. *B. thuringiensis (Bt) – грампозитивні спороутворюючі аеробні або факультативно анаеробні бактерії, здатні в процесі споруляції формувати видоспецифічні кристалоподібні включення білкової природи, які складаються з особливих термолабільних -ендотоксинів. Серологічні варіанти Bt продукують різні ентомотоксини, синтез їх багато в чому залежить від умов культивування культури. Накопичено багатий фактичний матеріал щодо походження ентомотоксинів, умов синтезу, будови, складу, токсичних властивостей і механізмів дії на комах. Bt набули домінантного положення у мікробіометоді боротьби із шкідниками рослин і тварин. На сьогодні існують різновиди Bt у більш ніж 70 варіантах (серотипах), вибірково специфічних до певного кола хазяїв-комах. Однак опис нових різновидів не завжди є виправданим з точки зору філогенетичної систематики, основаної на фенотипових ознаках.* ***Методи***. *На базі клонованих генів 16S рРНК ентомопатогенних бактерій BtH1, BtH10, BtH14 здійснено порівняльний філогенетичний аналіз внутрішньовидових взаємозв'язків Bt.* ***Результати***. *Прослідковано філогенетично однорідні лінії (рівень схожості 16S рРНК штамів 1-го і 10-го серотипів становить від 90,0 до 94 %; чіткого генетичного виокремлення штамів 10-го і 14-го серотипів не визначено).* ***Висновки***. *При порівнянні нуклеотидних послідовностей за 16S рРНК встановлено існування штамового поліморфізму всередині групи ентомопатогенів BtH1, BtH10, BtH14, пов'язаного з їхньою ентомоцидною активністю.*

Ключові слова: сероваріанти, Bacillus thuringiensis, філогенетичний аналіз.

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