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PHYLOGENETIC ANALYSIS OF OUTER MEMBRANE PROTEIN FAPA PRESENT IN DIFFERENT BACTERIA

The microorganisms, fungi and plants have evolved strategies to scavenge and absorb iron from soil, fresh and marine water, and living organisms (plants and animals). One of the most common strategies for iron accumulation is siderophore production. Siderophores are low-molecular-weight compounds (500–1500 daltons) possessing a high affinity and selectivity for iron(III). The biosynthesis of siderophores is typically regulated by the iron levels of the environment where the organism is located.

A large proportion of bacterial siderophore uptake studies have been centered on enteric bacteria, as typified by *Escherichia coli* and *Salmonella typhimurium*.

Key words: siderophores, protein FepA, phylogenetic analysis.

The aim of the research was to compare the results of phylogenetic analysis of amino acid sequence of the outer membrane protein FapA in different bacteria. As is known from the publication "Chemistry and biology of siderophores" by Robert C. Hider and Xiaole Kong [1], the outer membrane proteins, FecA, FepA, FhuA and FpvA *(Pseudomonas sp.)* have been characterized by X-ray crystallography and shown to be homologous structures with molecular weights in the region of 80 000. It is also known different proteins are selective for particular iron complexes, for instance, FepA (ferric–enterobactin permease) is selective for iron–enterobactin. FepA is an 81,000-dalton *E. coli* outer membrane protein that functions in the initial step of iron uptake by binding ferrienterochelin. It is also known that this protein is involved in the transport of pyoverdine, *P. putida* W619 [2].

In this connection it was interesting to see the presence of this protein in other bacteria and to distinguish the phylogenetic connections between them.

Materials and methods

We used annotated in Uniprot database [http://www.uniprot.org/] to obtain amino acid sequences of the protein FepA [3]. There were analyzed 80 sequences. The sample was formed by ignoring repeated releases of the sequences and avoiding excessive inclusion of the same microorganisms.

For phylogenetic analysis, consistently used local programs ClustalW and MEGA6. Clustal X is a windows interface for the ClustalW multiple sequence alignment program. It provides an integrated environment for performing multiple sequence and profile alignments and analysing the results. MEGA6 automatically infers the evolutionary tree by the NeighborJoining (NJ) algorithm that uses a matrix of pairwise distances estimated under the Jones–Thornton–Taylor (JTT) model for amino acid sequences [http://www.megasoftware.net/].

Results

As can be seen from Figure 1, 80 selected protein sequences can be grouped into several clusters, each of which is highly likely to form an internal node.

Cluster 1 have been combined *gammaProteobacteria* represented by *Enterobacteriaceae* family. Basically, this are gram-negative bacteria related to the genera *Citrobacter* and *Salmonella.*

In the Cluster 2 were also grouped *gammaProteobacteria* represented by *Enterobacteriaceae* family. However, in this case, most of the microorganisms in this cluster were representatives of the *Shigella* and *Escherichia* genera.

In the Cluster 3 have been combined gram-negative bacteria related to the genera *Enterobacter* and *Klebsiella.*

The most diverse in composition was Cluster 4, which we conditionally divided into 4 subclusters (Fig. 1). The probability of forming an internal node was 82%, which indicates a correct topology of the winds and nodes of the phylogenetic tree. Within the cluster, the probability for internal nodes was smaller and ranged from 50 to 12 %% (Fig, 1).

The combination of microorganisms in Cluster 4 are interesting. So in subcluster 4.1, have been combined according to the results of phylogenetic reconstruction, representatives of genera *Agrobacterim, Candidatus, Pseudoalteromonas, Pseudomonas, Kangiella, Arenibacter, Janthinobacterium.*

Subcluster 4.2 was mainly composed of representatives of genera *Bordetella, Xanthomonas, Pectobacteriom.*

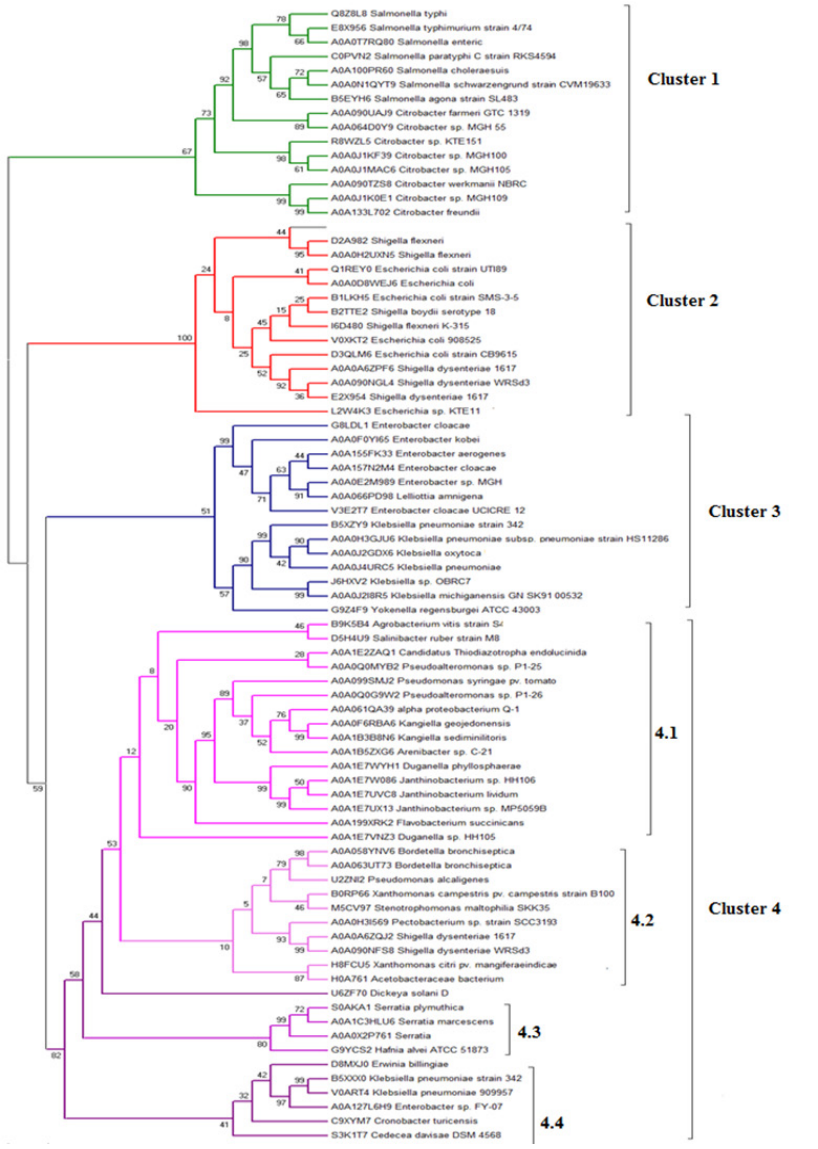

Fig. 1. Phylogenetic tree of the 80 amino acid sequence of protein FepA that been received from Uniprot database. The tree shown was obtained by NJ using Jones–Thornton–Taylor (JTT) model. Symbols on branches show bootstrap values with both the NJ (1,000 replicates)

Rarely mentioned genus *Serratia* and *Hafnia* was assigned to subcluster 4.3. They both belong to the *Enterobacteriaceae* family, and are gram-negative, facultatively anaerobic rod-shaped bacteria.

The biggest doubt was caused by subcluster 4.4, which include representatives of the genera *Enterobacter* and *Klebsiella* on a par with the representative of the genus *Cronobacter.* Previously, most of the amino acid sequences of the protein FepA defined for representatives of these species were assigned to Cluster 3, with the probability of forming an internal node of 53 % (Figure 1).

Based on the results of the study, it can be said that the amino acid sequence of the FepA protein for most microorganisms is indeed highly homologous. The presence of this protein is most famously characteristic of microorganisms belonging to the *Enterobacteriaceae* family.

References

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