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SHORT COMMUNICATIONS

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## Cometabolic Activity of Aphid *Acyrtosiphon pisum* and Symbiotic Bacterium *Buchnera aphidicola* APS

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Symbiosis is widespread in nature and occurs in many groups of plants and animals. Endocytobiosis is a particular case of symbiosis where a symbiont lives within the host cell. Endocytobiosis increases biodiversity and generates organisms with new properties, which ensure their life under extreme conditions [1].

Many aphids (Hemiptera: Aphididae) feed on phloem sap, as other insects of the order Homoptera [2]. Since sap contains only 20% of the essential amino acids necessary for the normal growth and living activity of aphids [3], aphids have to live in symbiosis with endocytobiotic bacteria of the genus *Buchnera*, which provide their host with nutrients present in insufficient amounts in the aphid diet. Bacteria occupy aphid cells known as bacteriocytes [4].

With the exception of methionine synthesis, *B. aphidicola* APS, which is a symbiont of aphid *Acyrtosiphon pisum*, synthesizes all amino acids essential for the insect, including arginine, valine, leucine, isoleucine, lysine, threonine, histidine, phenylalanine, and tryptophan [5].

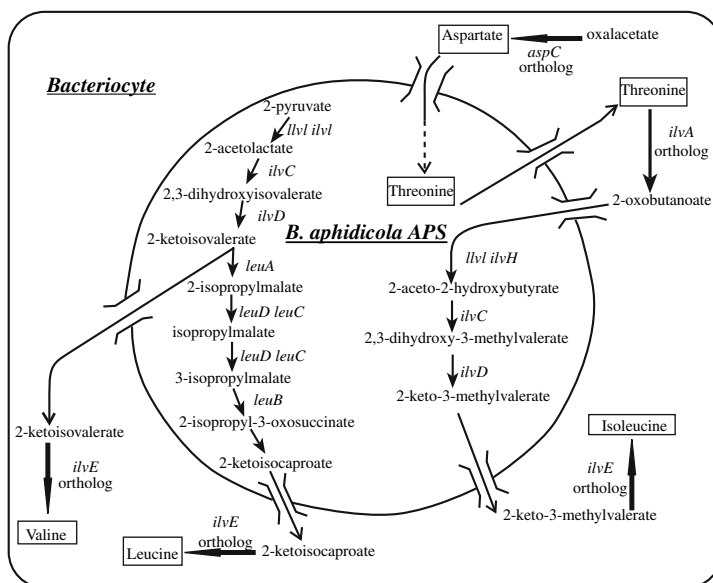
Using the GenomeExplorer program [6], we searched the *B. aphidicola* APS genome for the genes that are responsible for the function of the relevant metabolic pathways and compared the genes with those involved in the same metabolic pathways of *Escherichia coli*, which belongs to the same family Enterobacteriaceae as *Buchnera* does.

The orthologs of each gene were sought in the target genome by isolating the best two-sided match. The *B. aphidicola* APS genome proved to lack *ilvE*, *ilvA*, and *aspC*, whose products are involved in several reactions of the valine, leucine, isoleucine, and aspartate syntheses. However, the ultimate product of these metabolic pathways does occur in aphids [6].

A possible explanation of this paradoxical finding is that the missing genes occur in the host insect genome and that steps of amino acid syntheses are divided between the symbiont and the host. A comparative genome analysis showed that the valine, leucine, isoleucine, and aspartate synthesis enzymes whose genes are lacking in the *B. aphidicola* APS genome are involved in the final steps of valine, leucine, and isoleucine syntheses (*ilvE*), the first reaction of aspartate synthesis (*aspC*), and the first reaction of isoleucine synthesis from threonine (*ilvA*) (figure).

To check the above assumption, the *E. coli* proteins that catalyze the reactions lacking in *B. aphidicola* APS, control proteins, and proteins that catalyze the other reactions of the same pathways were compared with the draft version of the *A. pisum* genome (<http://www.ncbi.nlm.nih.gov/Tracer/>) with the BLAST program [7]. Indeed, the missing genes (*ilvE*, *ilvA*, and *aspC*) and only they had orthologs in the *A. pisum* genome; their function was confirmed by comparisons with GenBank. To verify the results, the genes were compared with the complete *Drosophila melanogaster* genome by using the BLAST program [7]. The gene set in question was found in the *D. melanogaster* genome.

The presence of the three genes in the insect genomes may be explained by the fact that *ilvE* and *aspC* are responsible for reversible transamination and, possibly, are necessary for controlling the relative levels of valine, leucine, isoleucine, and aspartate and redistributing the nitrogen pool in all insects. The *ilvA* gene is responsible for the irreversible conversion of threonine to 2-oxobutanoate, but it is still unclear if insects may need 2-oxobutanoate in appreciable amounts.



Possible division of the reactions involved in valine, leucine, isoleucine, threonine, and aspartate syntheses between *B. aphidicola* APS and *A. pisum*. The pathway of threonine synthesis from aspartate is shown with a dashed arrow.

We assume that, when *A. pisum* came to live in symbiosis with *B. aphidicola*, the latter became responsible for the majority of reactions involved in valine, leucine, isoleucine, and aspartate syntheses, while the reactions catalyzed by the products of *ilvE*, *ilvA*, and *aspC* remained involved in *A. pisum* metabolism.

A similar allocation of synthetic reactions is known for leucine biosynthesis in yeasts, whose *Leu4* is responsible for the first reaction in leucine synthesis and codes for two  $\alpha$ -isopropylmalate synthase isoforms. A long isoform is localized in the mitochondrial matrix, while a short isoform occurs in the cytoplasm. Both of the isoforms are involved in  $\alpha$ -isopropylmalate synthesis [8].

We studied whether a role is played by transmembrane proteins that are responsible for transporting substances from *B. aphidicola* APS cells into the bacteriocyte space. Information on the 14 transport proteins known for *B. aphidicola* APS was retrieved from TransportDB (<http://www.membranetransport.org/>). Of these proteins, YaiR, YnfM, Mdl, and MldB are potential candidates for this role, since they act as multisubstrate transporters. The other transporters have a narrow specificity for structurally different substances.

It was earlier believed that all reactions of amino acid syntheses occur in symbiotic bacteria. However, our findings indicate that some reactions occur in the host insect, while some others take place in symbiotic bacteria.

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