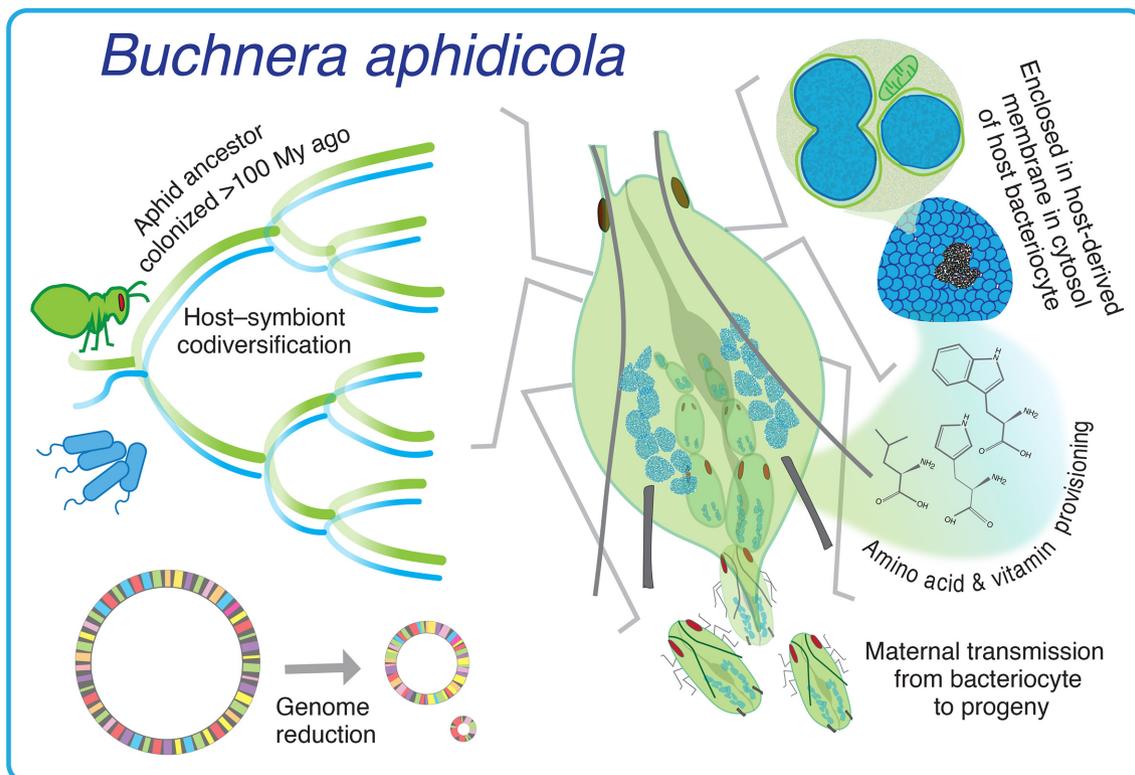


Microbe Profile: *Buchnera aphidicola*: ancient aphid accomplice and endosymbiont exemplar

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Graphical abstract

The obligate symbiont *Buchnera aphidicola* lives within cytoplasm of aphid bacteriocytes, where each symbiont cell is surrounded by a host-derived membrane. *B. aphidicola* undergoes strict maternal transmission and has codiversified with aphid host lineages for over 100 million years. Its clonal replication has resulted in extensive gene loss and accelerated protein evolution. Despite genome reduction, it retains genes for biosynthesis of essential amino acids, which are needed by the aphid host due to the lack of these nutrients in the phloem sap diet.

Abstract

Buchnera aphidicola is an obligate endosymbiont of aphids that cannot be cultured outside of hosts. It exists as diverse strains in different aphid species, and phylogenetic reconstructions show that it has been maternally transmitted in aphids for >100 million years. *B. aphidicola* genomes are highly reduced and show conserved gene order and no gene acquisition, but encoded proteins undergo rapid evolution. Aphids depend on *B. aphidicola* for biosynthesis of essential amino acids and as an integral part of embryonic development. How *B. aphidicola* populations are regulated within hosts remains little known.

TAXONOMY

Phylum: Proteobacteria; class: Gammaproteobacteria; order: Enterobacterales; family: *Erwiniaceae*; genus: *Buchnera*; species: *Buchnera aphidicola*. The species name is used for this clade of symbionts across all aphids; host names are appended to designate strains [1]. The type strain is *B. aphidicola* (*Schizaphis graminum*). The name honours Paul Buchner who studied endosymbionts using microscopy.

PROPERTIES

B. aphidicola has not been grown in laboratory culture. It only lives within the cytoplasm of specialized cells (bacteriocytes) of aphids (Insecta: Aphidoidea), enclosed by a host membrane. Most strains can produce all 10 essential amino acids and supply them to hosts, which feed on plant phloem, a diet low in these nutrients [2]. *B. aphidicola* cells are ~3.0µm diameter nonmotile spheres that produce flagellar basal bodies, which likely function in the secretion of peptides. Transmission to progeny occurs within the maternal body cavity: symbiont cells are exocytosed from maternal bacteriocytes and endocytosed by specialized cells of developing embryos [3].

GENOME

The first published endosymbiont genome for *B. aphidicola* (*Acyrtosiphon pisum*) was remarkable for its tiny size, consisting of a 0.64Mb main chromosome plus two small plasmids with genes for biosynthesis of tryptophan and leucine [4]. Also notable was the lack of novel (orphan) genes and the presence, despite drastic genome reduction, of pathways for biosynthesis of essential amino acids, which are limited in the phloem sap diet of aphids. With the sequencing of further *Buchnera* genomes, comparative analyses revealed that gene synteny is conserved, and that lineages undergo loss of genes but no gene acquisition [5]. Repetitive elements are lacking. Based on available *B. aphidicola* genomes representing 67 aphid species, sizes range from 0.41 to 0.65Mb and encode 354 to 587 protein-coding genes. G+C% ranges from 19–27% [6]. Each rRNA genes is single copy, with the 23S rRNA separate from the 16S-5S rRNA operon. In some cases, leucine and tryptophan biosynthetic genes are located on the main chromosome. *B. aphidicola* genomes are assembled from metagenomic samples, including host DNA in which *B. aphidicola* genome copies are 5–10× more numerous than those of hosts. Each *B. aphidicola* cell can contain 100 or more genome copies.

PHYLOGENY

In 1989, *B. aphidicola* became the first endosymbiont for which the 16S rRNA gene was sequenced, enabling phylogenetic analyses that showed its placement in the Gammaproteobacteria,

near *Escherichia coli*. Further sequencing of 16S rRNA genes for *B. aphidicola* from multiple aphid species showed that the phylogeny of *B. aphidicola* mirrors that of aphids, implying that an aphid ancestor was colonized at least 100 million years ago by an ancestor of *B. aphidicola*, and that symbiont and host subsequently codiversified. This pattern of parallel phylogenies of symbiont and host was further supported and extended by analyses based on whole-genome sequences from many aphid species. Compared to its free-living relatives, *B. aphidicola* shows accelerated sequence evolution, causing it to form long branches on phylogenetic trees. This obscures its exact placement within Gammaproteobacteria, although it is clearly related to *Enterobacteriaceae*.

KEY FEATURES AND DISCOVERIES

B. aphidicola is the best studied example of a widespread form of endosymbiosis found in many insects and some other invertebrate hosts. It provisions essential nutrients to hosts, with which it shares a long evolutionary history, such that it has become incorporated as an integral part of embryonic development. The sequestration of symbionts within individual hosts prevents recombination, resulting in strictly clonal replication. This clonality, plus small genetic population sizes due to bottlenecks during inoculation of progeny, results in the fixation of deleterious mutations due to genetic drift. One result is more frequent fixation of mutations causing amino acid changes, resulting in rapid protein evolution. Other changes cause the inactivation of non-essential genes, which then decay due to ongoing deletions of DNA, resulting in overall genome reduction. Many genes involved in cell wall biogenesis have been lost.

A notable feature of *B. aphidicola*, shared with many endosymbionts, is the constitutively high expression of heat shock proteins, including GroEL and DnaK. This elevated chaperone expression is hypothesized to counteract the low thermal stability of proteins that have accumulated many destabilizing amino acid replacements.

Changes in the *B. aphidicola* genome can directly affect the physiology and fitness of aphids. For example, mutations affecting the expression of the small heat shock protein IbpA can cause pea aphids to be extremely heat sensitive, but to enjoy a fitness advantage under constant cool conditions [7]. Other examples include inactivations of amino acid biosynthetic genes, which may be neutral when the aphid diet is rich in nutrients, but which are highly deleterious when the diet is limiting.

In some aphid species, *B. aphidicola* is joined by an additional bacterial endosymbiont, also transmitted vertically but confined to distinct bacteriocytes. In these cases, *B. aphidicola*

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Abbreviations: Mb, megabase pairs.

may lose some of the pathways for biosynthesis of amino acids or vitamins, which are instead supplied by the new endosymbiont [8]. In a few aphid lineages, *B. aphidicola* is entirely lost, and appears to be replaced by a novel bacterial or fungal endosymbiont.

For their part, aphids have evolved distinct capabilities for supporting or controlling *B. aphidicola* [9]. Bacteriocytes show distinctive gene expression patterns reflecting their roles as amino acid biosynthesis factories [10]. Additionally, aphid genomes have acquired several genes via horizontal transfer from bacteria other than *B. aphidicola*, and these are specifically expressed in bacteriocytes. Some of these function in recycling of peptidoglycan components and thus appear to contribute to regulation of *B. aphidicola* populations within hosts.

OPEN QUESTIONS

Despite being the most-studied insect endosymbiont, many mysteries remain regarding *B. aphidicola*. Here are several central questions that remain to be addressed.

- (1) Given that genes encoding classic amino acid transporters are largely missing from the *B. aphidicola* genome, how are amino acids transported from the host into the *B. aphidicola* cytoplasm, and how are essential amino acids exported to the host haemolymph?
- (2) How do the host and symbiont jointly regulate the population of *B. aphidicola* within an individual host so as to support progeny production and continued fitness of both host and symbiont?
- (3) What is the role of the flagellar basal bodies and, specifically, what peptides are secreted by these structures?
- (4) In some cases, genes considered to be essential, based on studies in model bacteria such as *E. coli*, are lost from the *B. aphidicola* genome: how are basic cellular processes maintained without these genes?

- (5) Can *B. aphidicola* be used as a specific target in strategies for the control of aphid populations that are agricultural pests?

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Conflicts of interest

The author declares that there are no conflicts of interest.

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