

New complete genome of the *Bartonella apihabitans* strain EK0718, obtained from Kazakhstan

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ABSTRACT

Background/Objectives: *Bartonella apihabitans* strain EK0718 is a notable nonpathogenic member of the *Bartonella* genus, distinguished by its absence from the core components of the bee microbiome. This highlights its unique role and the complexity of microbial interactions within bee ecosystems. **Methods:** In this study, we present the new draft genome of the *B. apihabitans* strain EK0718 from bees, generated from Illumina reads using the SPAdes assembler. **Results:** The assembled genome has a total size of 2.643257 Mb. Annotation of the *B. apihabitans* strain EK0718 genome assembly revealed 2,305 genes, including 2,248 protein-coding genes and 57 RNA genes. **Conclusions:** This genome serves as a valuable resource for enhancing our understanding of *Bartonella* genetics and evolution, as well as studying how microorganisms evolve from nonpathogenic to pathogenic forms.

Keywords: Bee, *Bartonella*, Complete genome.

Article type: Report.

INTRODUCTION

Numerous bacterial pathogens that exclusively infect a narrow range of host organisms and are responsible for chronic infectious diseases have emerged from free-living symbiotic ecological ancestors. This transformation is the result of a gradual yet powerful evolutionary process that enables these pathogens to adapt effectively to their hosts (Moran *et al.* 2008; Sachs *et al.* 2011; Weiland-Bräuer 2021). This process is marked by significant evolutionary changes in the symbiotic microorganism. One of the most striking transformations is a reduction in genome size, driven by a limited dietary diversity because of the narrower range of host organisms it interacts with. Furthermore, these microorganisms often gain virulence factors, enhancing their adaptability and survival in specific environments (Moran 2002; Wolf & Koonin 2013; Nakayama *et al.* 2025). The study of such changes associated with lifestyle changes provides a unique window into understanding the ecology and evolution of not only pathogens but also their symbiotic precursors (Gupta & Gaur 2025). To this end, obtaining the complete genome of a non-pathogenic microorganism could provide valuable information, especially since the diversity of non-pathogenic variants from insects continues to increase (Zientz *et al.* 2001; Land *et al.* 2015; Schadron 2024). In our research, the complete genome of a representative of the genus *Bartonella* was obtained from a sample of dead bees in Kazakhstan using shotgun sequencing of the sample. *Bartonella* is the type genus of the Bartonellaceae family, which belongs to the Alphaproteobacteria class. The genus *Bartonella* currently has 39 characterized species and three subspecies (Luo *et al.* 2023). *Bartonella* species are well known for infecting erythrocytes and endothelial cells in a variety of mammals, including humans, cats, dogs, ruminants, wild rabbits, and wild rodents (Álvarez-Fernández *et al.* 2018; Luo *et al.* 2023). A comparative analysis of various representatives of the genus revealed that the tree has four branches, indicating that symbionts evolved into

pathogens. It has been suggested that the ancestor of pathogenic *Bartonella* was an insect gut symbiont (Segers *et al.* 2017). Phylogenomic analysis of isolates has revealed several clusters within the genus *Bartonella* (Liu *et al.* 2022; Alsarraf *et al.* 2025), suggesting the presence of multiple *Bartonella* species in the honeybee gut. The colonization of blood-sucking arthropods is thought to be a critical step in the evolution of gut symbionts into pathogens (Segers *et al.* 2017; Sonenshine & Stewart 2021).

MATERIALS AND METHODS

Samples

The work used dead bees obtained from several apiaries in Kazakhstan (BioSample accession SAMN53323920, BioProject ID PRJNA1366779). For isolation, DNA material was immediately frozen and stored at temperatures ranging from -90 °C to -15 °C.

DNA isolation, genome sequencing, assembly, and annotation

Genomic DNA was isolated using the PureLink Genomic DNA Mini Kit according to the manufacturer's instructions (ThermoFisher Scientific, Waltham, MA, USA). A whole-genome sequencing library was prepared using the Nextera XT DNA library preparation kit following the manufacturer's instructions (Illumina, Cambridge, UK). The libraries were sequenced with the Miseq platform (Illumina, Cambridge, UK) to generate 2 × 300 paired-end reads. The raw reads adapters were trimmed by Trimmomatic version 0.38.0 (Bolger *et al.* 2014). Lower-quality sequences (< Q30) were removed. After trimming, reads ranged from 50 to 250 bp. Genome assembly was done with SPAdes version 3.12.0 (Bankevich *et al.* 2012). The draft sequences were filled with gaps using GapCloser v1.12 (Xu *et al.* 2020), and the genome was assembled into a single scaffold. Following assembly, the genome's quality was assessed using the software Geneious Prime 2023 by mapping to the reference genome (Kearse *et al.* 2012). The annotation genome was determined using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP), GeneMarkS-2+, RAST, and Bacta (Tatusova *et al.* 2016). The predicted proteins were classified into KEGG categories, and metabolic pathways were identified using KEGG Mapper (Kanehisa & Sato 2020). Additional analysis was carried out with MicrobeAnnotator (Ruiz-Perez *et al.* 2021). The biosynthetic gene clusters for secondary metabolites in the strain EK0718 draft genome were identified using antiSMASH version 7.0. (Blin *et al.* 2023).

Phylogenomics and molecular identification

The whole-genome and RNA polymerase B unit sequences of *Bartonella* were analyzed. RNA polymerase B unit sequence selection was extended to the RNA polymerase B unit of bacteria identified based on their high similarity using NCBI BLAST 25 (Altschul *et al.* 1990). Sequences were aligned by MAFFT from Geneious Prime 2023 (Kearse *et al.* 2012), and a maximum likelihood phylogenetic tree was inferred by bootstrapping (600 replicates). Thirty-three *Bartonella* sequences were analyzed for the closest matches to reference nucleotide sequences in the GenBank database using the NCBI Nucleotide BLAST tool. All sequences were validated, aligned, and compared for genetic similarity using MegAlign (Geneious Prime 2023).

RESULTS

Genome characterization of *Bartonella apihabitans* strain EK0718

As a result of sequencing samples of dead bees collected in Kazakhstan (BioSample accession SAMN53323920, BioProject ID PRJNA1366779) and assembly of the *Bartonella apihabitans* genome, a complete genome of the microorganism was compiled, the main characteristics of which are presented in Table 1. The genome contained 8,548,838 base pairs. Genome annotation revealed a total of 2,305 genes, which include 2,248 protein-coding genes, 57 RNA genes, and 28 subsystem sequences (Fig. 1).

Table 1. Characteristics of genome assembly of *Bartonella apihabitans* strain EK0718.

Characteristic	Quantity
Total genome size, bp	2643257
Genes (total)	2,305
CDSs (total)	2,248
Genes (coding)	2,195
CDSs (with protein)	2,195
Genes (RNA)	57
tRNAs	47
ncRNAs	4

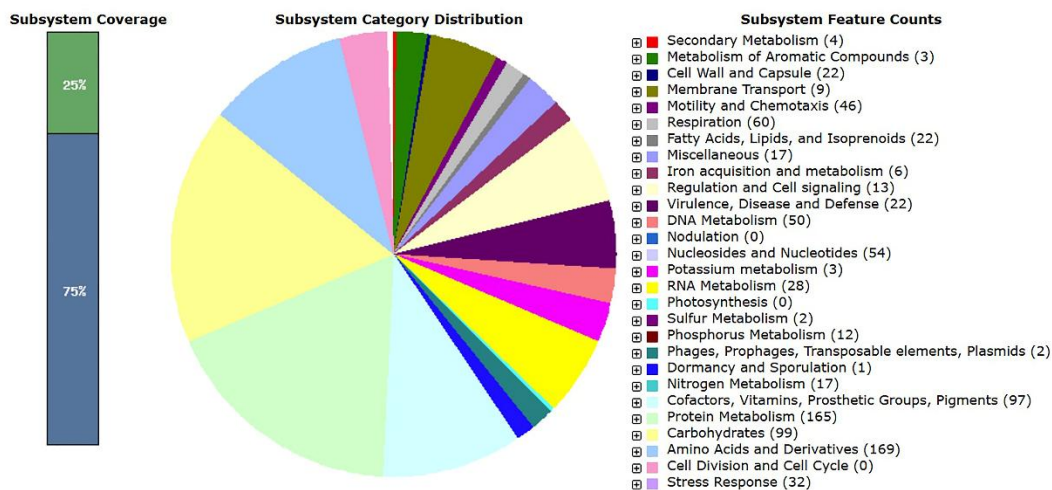


Fig. 1. Subsystem statistics information of *Bartonella apihabitans* strain EK0718 using RAST annotation. The subsystems category and corresponding feature counts were shown in the legend.

Genome annotation was carried out using the automated RAST (Rapid Annotations using Subsystems Technology) platform, which was integrated into the SEED Viewer environment. The analysis of the *B. apihabitans* strain EK0718 genome enabled us to characterise the structure of the functional annotation and identify key metabolic pathways represented in the genome. The annotation results show that approximately 25% of the genes were assigned to known functional subsystems, while approximately 75% remained outside the subsystem approach. The largest and most metabolically significant clusters, which unite about 100 or more genes, are: amino acids and derivatives (169 genes), protein metabolism (165 genes), carbohydrates (99 genes), cofactors, vitamins, prosthetic groups, and pigments (97 genes). Subsystems associated with energy metabolism (respiration, 60 genes), DNA metabolism (50 genes), nucleotide and nucleoside metabolism (54 genes), and chemotaxis (46 genes) represent smaller but equally important functional blocks. Of particular interest in the context of *Bartonella* pathogenesis are the subsystems associated with virulence and host interaction. In the studied genome, these include 22 genes related to "Resistance to antibiotics and toxic compounds" and "Invasion and intracellular resistance." These genes represent universal stress response and defense mechanisms that ensure resistance to natural antimicrobials, reactive metabolites, and intestinal conditions. Photosynthesis, secondary metabolism, aromatic compound metabolism, and iron, potassium, and sulphur metabolism are among the subsystems that are missing or under-represented in the genome under study. Thus, the functional structure of the *B. apihabitans* genome is defined by a combination of basic metabolic pathways required for bacterial viability and those characteristic of bacteria adapted to life in the gut environment of honeybees. During the functional characterization of the microorganism genome, the possibility of the existence of 4 clusters for the synthesis of secondary metabolites was noted. The secondary metabolite biosynthetic gene clusters of the strain EK0718 draft genome were identified with antiSMASH version 7.0 (Fig. 2). The detected secondary metabolites prevent the destruction of the microorganism by external adverse factors.

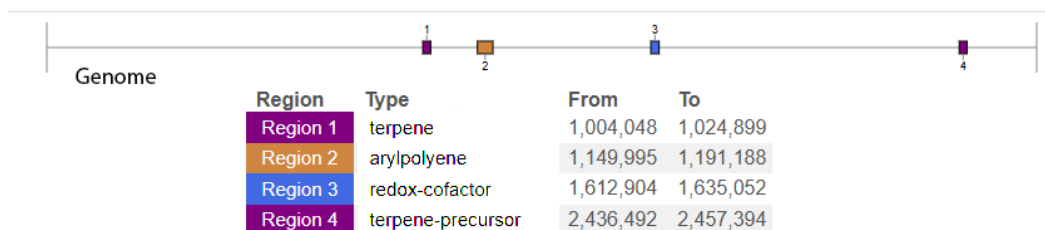


Fig. 2. Location of secondary metabolite biosynthesis gene clusters across the genome *Bartonella apihabitans* strain EK0718.

Thus, the presence of a significant proportion of genes not assigned to subsystems emphasizes the need for additional functional annotation and may reflect unique mechanisms of interaction with the host, unique to the genus *Bartonella*.

Phylogenomics and molecular identification

A phylogenetic analysis of the assembled *Bartonella* genome was conducted using the RNA polymerase subunit model, employing thirty-three protein sequences sourced from the NCBI database. Fig. 3 clearly demonstrates that all representatives of this genus are categorised into several distinct groups.

Two of these groups are pathogenic, while the other two serve as transitional forms between arthropods and mammals. Additionally, there is one group comprised of symbiotic microorganisms associated with bees. The phylogenetic analysis decisively distinguishes the nonpathogenic *Bartonella* group into two subcategories: European and American strains. Notably, the genome under investigation is poised to represent a third group of *Bartonella* strains that thrive in the intestines of bees.

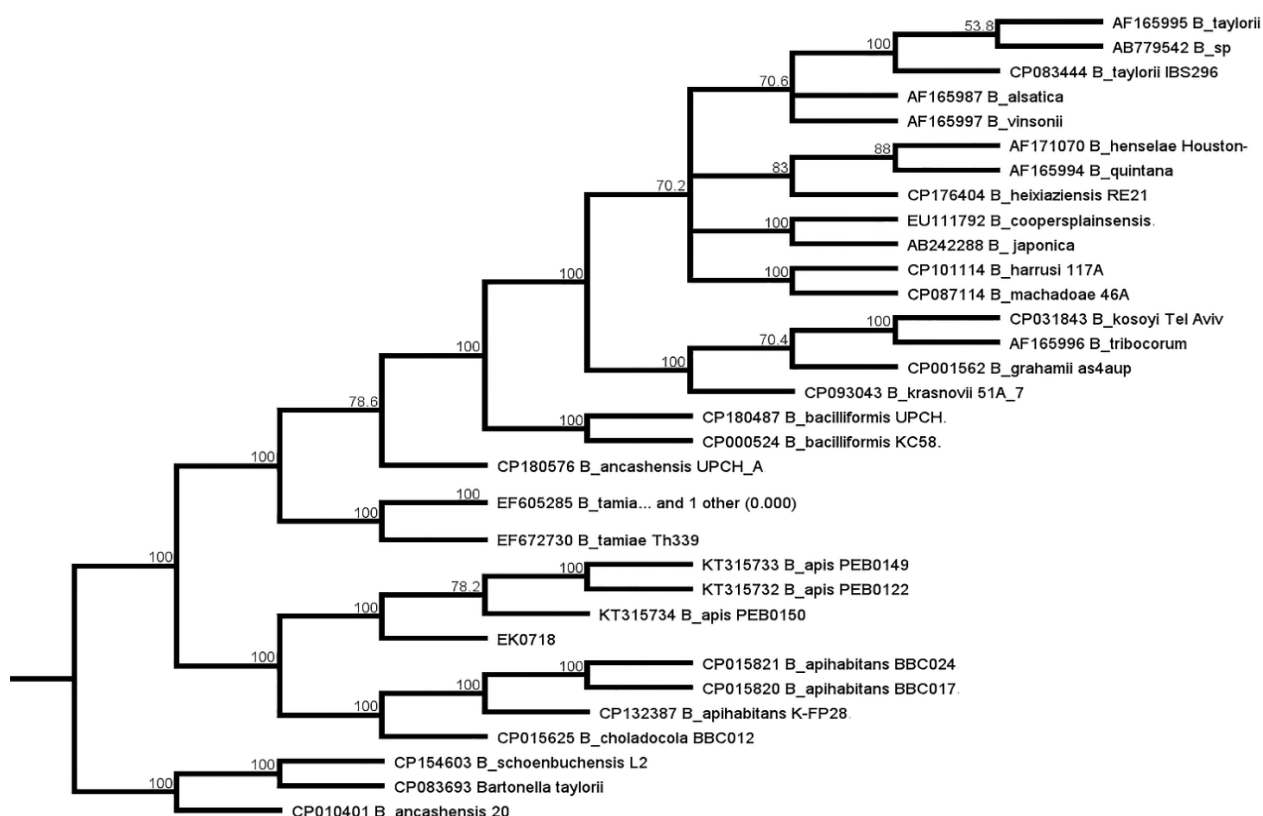


Fig. 3. Phylogenetic analysis of *Bartonella* strains on the model of RNA polymerase B unit gene.

Comparative KEGG studies of the genes of *Bartonella apihabitans* strain EK0718, *B. apihabitans* strain BBC0178 (CP015820), and *B. choladocola* strain BBC0122 (CP015625) were performed to further investigate the relationship between the presented genome and typical representatives of bee *Bartonella*. The presented genome was found to be evolutionary intermediate between existing *Bartonella* strain groups (Fig. 4).

While it shares similarities with *B. apihabitans* strain BBC0178 in terms of tryptophan, isoleucine, cobalamin, thiamine, betaine, and other biosynthesis pathways, it differs from *B. choladocola* strain BBC0122 (CP015625) in terms of molybdenum cofactor synthesis, purine degradation, and nitrate reduction. When comparing the genes for the Krebs cycle and pyruvate reduction, the presented genome stands out. Thus, the presented *Bartonella* strain genome was found to differ from the two other strain groups registered with NCBI.



Fig. 4. KEGG genes comparing the presented genome to typical *Bartonella* bee representatives.

DISCUSSION

The core bacterial gut microbiota of honey bees is relatively stable, consisting of five to eight bacterial taxa with specialized metabolic capabilities (Gaggia et al. 2023). Environmental or rearing conditions, such as seasonality (Kešnerová et al. 2020), diet and feed additives (Gaggia et al. 2023), xenobiotics (Motta et al. 2020) or pathogens (Jabal-Uriel et al. 2022). The proportions of major microbial genera, or their presence/absence, have a direct impact on gut microbiome functionality, influencing honeybee behavior via disruption of the gut-brain axis (Liberti et al. 2022) and nutrient absorption efficiency (Ansaloni et al. 2025). Thus, the relationship between the microbial population and the environment represents a new frontier in our understanding of the honeybee microbiome's structure and function. We present a new complete genome of a representative of the genus *Bartonella* with quantitative seasonal changes in microbiome composition. The largest subsystem categories—amino acids and their derivatives, protein metabolism, carbohydrates and cofactors, vitamins, and pigments—represent essential metabolic groups necessary for survival in the bee gut environment, according to functional annotation of the *Bartonella apihabitans* EK0718 genome using the RAST/SEED platform. Other bee-associated

gut bacteria, such as *Snodgrassella* and *Gilliamella*, have similar functional profiles (Prasad *et al.* 2025). These bacteria rely on a variety of metabolisms of carbohydrates and amino acids to survive in the nutrient-rich but unpredictable environment of the insect gut (Prasad *et al.* 2025). Additionally, genes linked to respiration, DNA metabolism, nucleotide biosynthesis, and chemotaxis imply that *B. apihabitans* preserves the metabolic profile required for gut microbiome interactions. This metabolic profile aligns with the ecological function of *Bartonella* species in insects, which are frequently not strict pathogens, but rather facultative symbionts (Segers *et al.* 2017). The presence of 22 genes associated with virulence-related subsystems is particularly noteworthy. These genes are linked to antibiotic resistance, tolerance to toxic compounds, invasion, and intracellular survival. In non-pathogenic species of *Bartonella*, such genes typically serve functions related to stress adaptation, detoxification, and competition among microorganisms, rather than facilitating invasion of host tissues (Prasad *et al.* 2025). It is suggested that certain *Bartonella* species associated with bees may retain ancestral forms of virulence genes that have been repurposed to enhance their ecological fitness within the intestinal environment. This idea relates to their identification in the analyzed genome (Segers *et al.* 2017; Liu *et al.* 2025). Subsystems involved in photosynthesis, secondary metabolism, and the degradation of aromatic compounds, as well as the metabolic pathways for iron, potassium, and sulfur, were either absent or only minimally expressed. Similar reductions in gene numbers have been observed in other symbiotic bacteria, which tend to depend on the nutrient availability from their host and have streamlined genomes. The substantial proportion of genes unrelated to these subsystems underscores the necessity for further functional studies and may indicate specific adaptations of lineages that contribute to the ecological specialization of the bee microbiome. The microorganism's ability to directly participate in the bee's Krebs cycle by synthesizing pyruvate, as well as its ability to synthesize the essential amino acids tryptophan and isoleucine, which can support bees during the winter, when their diet is depleted, was of particular interest. A phylogenetic analysis revealed that the genome under investigation could serve as the foundation for the emergence of a new phyletic subgroup within the complex of *Bartonella* strains in the bee gut. This finding can be explained by Kazakhstan's geographic location, which has distinct periods of high and low temperatures, resulting in evolutionary changes in the genome of the symbiotic microorganism. Several unique characteristics of this representative of the genus *Bartonella* have been identified, which, with further study, will allow not only a better understanding of the changes in the intestinal microbiome of bees but also shed additional light on the mechanism of transformation of a symbiotic organism into a pathogenic one.

CONCLUSION

The article successfully sequenced and annotated the genome of a nonpathogenic *Bartonella* species, *B. apihabitans* strain EK0718, providing a foundation for future comparative and evolutionary studies, particularly regarding the development of pathogenicity.

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