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Proteomic and Electron Microscopy Study of Bacteriophages From *Bartonella Henselae* And *Bartonella Grahamii*

Research Article

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Abstract

Bacteriophages are one of the key factors playing an important role in exchange of genetic information between different bacterial species leading towards bacterial evolution. Their study becomes especially significant if bacteriophages contribute to the development of new human pathogens. In the present study we have investigated the occurence of bacteriophages in *Bartonella*, a genus of Gram-negative bacteria representing facultative intracellular parasites causing strong infections mainly in immune-compromised patients. Proteomic and morphologic characterization of bacteriophage preparations from B. *henselae* and B. *grahamii* bacteriophages indicated the presence of three different types of bacteriophages. Bacteriophage-like particles with diameter 42 nm, non-enveloped tailed bacteriophages and large enveloped phages with icosahedral to round cores were identified. Most of the results of our observations suggests, that *B. henselae* is the host of tailed dsDNA bacteriophages belonging to order *Caudovirales* and family *Myoviridae* (similar to bacteriophage P2), and enveloped bacteriophages similar to dsRNA viruses from family *Cystoviridae*. Small size bacteriophage-like particles could correspond to defective or satellite phages. Similar conclusions might be drawn for *B. grahamii*, though less experimental evidences are available.

Keywords: *Rochalimaea*, Cat Scratch Disease, Zoonotic Disease, Evolution of Pathogenic Bacteria, Horizontal Gene Transfer

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Introduction

Bartonella (known also as *Rochalimaea*) is a genus of Gram-negative bacteria representing facultative intracellular parasites. These bacteria can on occasion infect healthy people, but are considered as opportunistic pathogens causing strong infections in immune-compromised patients such as those with AIDS.

Bartonella-s are transmitted by blood sucking arthropods, e.g. ticks, fleas, sand flies and mosquitoes. They use zoonotic reservoir for their transfer to human. After their invasion of primary niche - endothelial cells of reservoir animal, they are released in period intervals to bloodstream, where they invade erythrocytes. Bacterial infection might be transferred to human through occasional scratch by infected animal.

Bartonella henselae is a zoonotic pathogen using cats as a reservoir and causing cat scratch disease (CSD) [1]. The disease has serious symptoms characterized by lymphadenopathy and persistent fever. It might have even more severe symptoms in AIDS patients, where it can cause bacillary angiomatosis, bacillary peliosis hepatis, endocarditis and bacteremia associated with relapsed fever [2]. *B. henselae* has been identified as a one of eyes infecting bacterial species occurring with increasing incidence [3,4]. Another species involved in intraocular inflammatory disease is *Bartonella grahamii*, which uses rodents as a reservoir [5]. Infection in this case triggers even behavioural changes in patients [6].

Bacteriophages are one of the key elements playing an important role in acquisition of new genetic information by various bacterial species leading towards evolution of bacteria [7]. Bacterial survival and adaptation to new environmental conditions and host species are likewise mediated by bacteriophages. Furthermore, they might enhance bacterial evasion or inactivation of host defense mechanisms. In addition, bacteriophage genomes often contain a variety of genes horizontally transferred to various bacteria by phage infection. Importance of our knowledge concerning bacteriophages increases, if they are carrier of genes encoding virulence factors. Phage infection raises virulence of the bacteria and

sometimes converts a non-pathogenic strain to a dangerous pathogen [8]. Thus, questions regarding evolution of bacteriophages, acquiring of new genes, their diversification and horizontal transfer between bacterial species are one of the issues with biological and medical relevance.

Several authors have demonstrated the occurrence of bacteriophage-like particles amongst *Bartonella-s* [9,10,11,12]. The particles conserved among different *Bartonella* species are capable to package host DNA and export it outside of host cells. It suggests possible role of these bacteriophage particles in genetic exchange [13].

The present study confirmed the occurrence of before reported bacteriophage particles in both *B. henselae* and *B. grahamii* with diameter approximately 40 nm and with similar morphology and relative molecular weights of three main bacteriophage proteins. Furthermore, large enveloped and non-enveloped tailed bacteriophages were found in both cultures as well. To characterize structural bacteriophage proteins, we have performed sensitive proteomic analysis and identified bacteriophage-associated proteins.

Materials and Methods

Growth of Bacterial Strains

B. henselae Houston-1 and *B. grahamii* were routinely grown for 5-10 days on blood agar containing 5 % (v/v) horse blood (CSB agar; Statens veterinärmedicinska anstalt (SVA), Uppsala, Sweden) in a humidified atmosphere with 5 % (v/v) CO_2 at 35°C.

Isolation of Bacteriophages

The isolation procedure was adopted from Anderson et al. (1994) [10] with a few additional modifications. Bacteria were scraped from agar plates and immediately transferred into sterile Dulbecco´s phosphate buffered saline (DPBS; pH 7,2). Bacterial biomass obtained by growth on each plate was collected by addition of 0,5 ml of DPBS. Bacteria were pelleted by twice repeated centrifugation for 1 min at 10000 × g. The supernatant after second centrifugation was collected and bacteriophages were precipitated by addition of the solution consisted of 20 % (w/v) PEG 8000 and 2,5 M NaCl. The mixture was incubated overnight at 4°C. Bacteriophage pellet was collected by centrifugation at 10 000 × g for 10 min. Then particles were suspended in sterile SM buffer [14] and left for a few hours on ice before complete resuspension. The bacteriophage suspension was further stored at 4°C.

SDS-PAGE

Bacteriophage suspension was solubilized in NuPAGE® LDS 4×LDS sample buffer (Invitrogen AB, Sweden), cleaned-up using ProteoExtract™ Protein Precipitation Kit (Calbiochem, Merck-4Biosciences, Sweden) and solubilized again in NuPAGE® LDS 4×LDS sample buffer. Sample was run at reducing conditions on 4-12 % NuPAGE Bis-Tris Gel using MOPS running buffer. The gels were stained by Bio-Safe Coomassie Stain (Biorad, Sweden), individual lanes cut off and used for proteomic analysis

Proteomic Analysis

Excised polyacrylamide slices have been further reduced, alkylated and in-gel digested by trypsin [15]. Nanoflow LC-MS/MS analysis was performed on a 7-tesla hybrid linear iontrap (LTQ) FT mass spectrometer (Thermo Electron, Bremen, Germany) modified with a nanoelectrospray ion source (Proxeon Biosystems, Odense, Denmark) according to the protocol of Nielsen et al (2005) [16]. Protein and peptides were identified by searching of tandem mass spectra using Mascot search engine against the non-redundant NCBI protein database. Analysis was performed twice and only statistically significant Mascot matches with individual ion scores indicating identity or extensive homology above cut off score for 95 % of correctly assigned peptides ($p \leq 0.05$) and at the same time existing in both analyses were selected. Only proteins identified by minimum two peptides were included in the study and the proteins assigned through single peptide matches were excluded from further analysis.

Transmission Electron Microscopy

Sample preparation was performed according to the protocol of Barbian and Minnick (2000) [11]. Bacteriophage suspension was deposited on Silicon Monoxide Type-A support grids (300 mesh copper) and stained by 1 % (w/v) sodium phosphotungstate. The grids were observed under Zeiss Supra 35VP electron microscope.

Results

To investigate occurrence of bacteriophages in the cultures of *B. henselae* (BH) Houston-1 and *B. grahamii* (BG), bacteriophages have been isolated from *in vitro* cultured bacteria. These preparations have been characterized by both morphologic and proteomic analysis.

Size and Morphology of Bacteriophage-like Particles in *B. Henselae* and *B. Grahamii*

In the current study, the electron microscopy images showed the presence of icosahedral particles in two ranges of sizes. In both BH and BG bacteriophage preparations, icosahedral particles having average diameter between opposite vertices equal to 42 nm were present (Fig. 1 A). The average value of the diameters between opposite faces was 36 nm (Fig. 1 A). Furthermore, in both bacterial cultures there were also observed larger enveloped bacteriophages with icosahedral to round shaped core (Fig. 1 B, C, D) and tailed bacteriophages (data not shown). Both detected enveloped and non-enveloped phages were larger in their diameters than before described 40 nm particles. The diameter of enveloped particles varied between 110-160 nm, while cores of enveloped particles had diameters in range between 88-110 nm. The tailed phages consisted of a capsid with average diameter 87 nm; tail and fibers were about 175 nm and 83 nm long.

Protein Analysis of *B. Henselae* and *B. Grahamii* Bacteriophages

SDS-PAGE analysis of BH bacteriophages showed several protein bands with following relative molecular weights: 21; 27; 30; 32; 42; 46 and 63 kDa, where major bands had Mr equal to 32; 46 and 63 kDa. Following values relative molecular weights of protein bands were detected by analysis of BG bacteriophage preparations: 14; 19; 25; 27; 30; 32; 33; 36; 37; 42; 45; 51; 60 and 63 kDa.

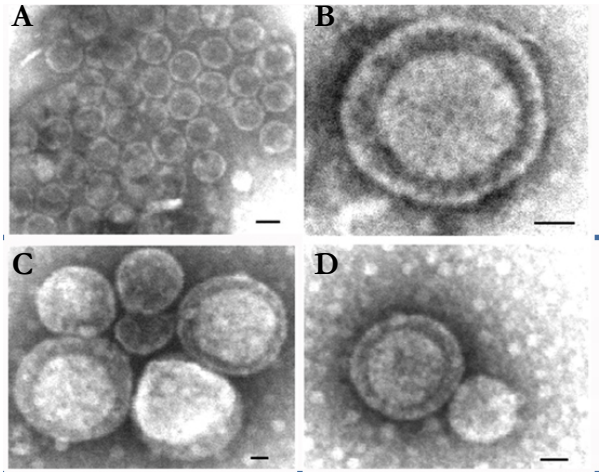
Further proteomic analysis using LC-MS/MS assisted in identification of several of these proteins and also included other

low-abundant proteins phage proteins. Results of this analysis including information concerning their potential functions are summarized in both Table 1 and Table 2.

Discussion

In our present study, bacteriophages of different types were detected by electron microscopy in the bacteriophage suspensions prepared from cultures of both BH and BG. By morphologic analysis we have identified before described bacteriophage-like particles, non-enveloped tailed and novel enveloped bacteriophages. The proteomic analysis of structural phage proteins has

Figure1. Bacteriophages isolated from cultures of facultative intracellular parasites *Bartonella grahamii* and *henselae*.



Electron microscopy of *B. grahamii* (A, B) and *B. henselae* (C, D) bacteriophages prepared by differential centrifugation followed by precipitation using 20 % (w/v) PEG/2.5 M NaCl. Bacteriophage suspension was deposited on Silicon Monoxide Type-A support grids and stained by 1 % (w/v) sodium phosphotungstate. Bacteriophage-like particles from *B. grahamii* (A) and large enveloped bacteriophages occuring in both *B. grahamii* (B) and *B. henselae* were observed (C, D) under Zeiss Supra 35VP electron microscope.
Scale bars: 30 nm

Table 1. The list of proteins identified by LC-MS/MS analysis of bacteriophage preparations from *B. henselae*. The proteins were identified by minimum two peptide matches and using statistically significant Mascot scores above threshold indicating 95% correctly assigned peptides (p≤0.05). Minimum Mascot score value of matches used in this study was 63.

Protein name	NCBI Accession number	Protein mass/aa	Motifs/E-value	Function	Other homologs/Accession number/E-value
Phage tail protein	CAF27115	8179/74	cl02088, Phage_ tail_X super family, Phage Tail Protein X/4.14e-17	P2-like phage tail protein	Phage tail protein [*Bartonella tribocorum*]/ YP_001608777.1/5e-45
Phage protein gp25 (Baseplate assembly protein W)	CAF27129	12171/109	cl01403, GPW_gp25, Gene 25-like lysozyme/ 3.33e-36	Structural protein of outer wedge of T4 baseplate having acidic lysozyme activity	Baseplate assembly protein W [*Bartonella washoensis*]/ WP_006925683/ 1e-32
Phage tail protein	CAF27116	14011/126	pfam06995, Phage P2 GpU/ 3.02e-49	P2 GpU protein probably involved in tail assembly	Tail formation protein, phage P2 GpU [*Pseudomonas fluorescens A506*]/ YP_006323125/1e-57
Phage-related protein	CAF27181	14389/127	cd03352, UDP-3-O-acyl-glucosamine N-acyltransferase (LpxD)/ 2.85E-4	Lipid A biosynthetic pathway in Gram-negative bacteria	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase [*Mannheimia succiniciproducens* MBEL55E]/ YP_089114.1/ 2e-24
Single-strand binding protein	CAF27165	16780/148	[COG0629], Single-stranded DNA-binding protein/4.12e-37	DNA replication, recombination, and repair	Single-strand binding protein (ssb) [*Bartonella tribocorum CIP 105476*]/ YP_001609352.1/1e-92
Major tail tube protein FII	YP_033153	18614/168	pfam04985, Phage tail tube protein FII/3.08e-68	Phage tail	Phage tail tube protein [*Bartonella tribocorum* CIP 105476]/ YP_001608942.1/7e-118
Phage protein	CAF27122	19607/173	cl10713, Phage pRha, Phage regulatory protein Rha/7.13e-20	Probable inhibition of bacterial host transcription	Phage protein [*Bartonella* sp. AR 15-3]/ CBI79586.1/5e-114
Anti-repressor protein	CAF27155	19738/171	[pfam08346], AntA/AntB antirepressor/3.07e-35	Bacteriophage anti-repressor	Anti-repressor protein [*Bartonella tribocorum* CIP 105476] / YP_001608925.1/6e-64

Anti-repressor protein	YP_033189	20697/180	[pfam08346], AntA/AntB antirepressor/8.51E-32	Bacteriophage anti-repressor	anti-repressor protein [*Bartonella tribocorum* CIP 105476 YP_001608925.1/1.e-83
Phage-related protein	CAF27489	21141/259	pfam04404, ERF, ERF superfamily/1.41e-20	DNA single-strand annealing protein, DNA recombination	Recombinase [*Bartonella taylorii*]/ WP_0107047847.1/2e-127
Phage protein gp13	CAF27147	21649/193	-	Structural protein of neck of bacteriophage T4	Phage protein [*Bartonella vinsonii*]/ WP_0107047847.1/ 2e-127
Phage-related baseplate assembly protein	CAF27130	24148/229	[pfam04717], Phage-related baseplate assembly protein/5.34e-21	Phage baseplate assembly	phage-related baseplate assembly protein [*Bartonella sp.* 1-1C]/ CBI80300.1/2e-140
Phage related lysozyme	YP_033722	24505/221	cd00737, endolysin, autolysin/2e-40	Enzyme of dsDNA phages hydrolysing beta-1,4-linked polysaccharides involved in bacterial cell wall	Lysozyme [*Bartonella sp.* DB5-6]/ WP_007552512.1/ 9e-135
Anti-repressor protein	CAF27154	28812/252	cl01430, AntA, AntA/AntB antirepressor/ 1.61e-36	Bacteriophage anti-repressor	Antirepressor [*Bartonella doshiae*]/ WP_004856719.1/ 7e-162/
31K major protein, Pap31	JC6528	30056/281	pfam02530, Porin_2, Porin subfamily/4e-34	Membrane channels for transport of hydrophilic compounds	Hemin binding protein [*Bartonella vinsonii*]/ WP_010704727.1/ 1e-135
Hemin binding protein c	YP_031945	30103/277	[COG3637], Opacity protein and related surface antigens/ 4.45e-25	Cell envelope biogenesis, outer membrane	Hemin binding protein c [*Bartonella quintana str. Toulouse*]/ YP_031945.1/0.0 Omp25/ropB family outer membrane protein [*Bartonella bacilliformis* KC583]/ YP_989466.1/ 5e-96
Hemin binding protein d	YP_033317	30251/274	[COG3637], Opacity protein and related surface antigens/ 5.42e-23	Cell envelope biogenesis, outer membrane	Hemin binding protein [*Bartonella vinsonii subsp. berkhoffii* str. Winnie]/ YP_007462349.1/ 1e-139
Phage protein gp26	CAF27128	30840/275	cl01294, Baseplate J-like protein/ 1.02e-35	Protein located on baseplate edge of bacteriophage P2,	Phage-related baseplate assembly protein [*Bartonella sp.* 1-1C]/ CBI80302.1/0.0
Phage protein gp20	YP_033172	40117/358	[pfam03864] Phage_cap_E/ 6.97e-87	Major capsid protein E of the phage heads, stabilisation of the condensed form of the DNA molecule	Phage protein [*Bartonella tribocorum* CIP 105476]/0.0
Phage protein gp18	CAF27143	40386/369	cd07022, Signal peptide peptidase A/ 3.00e-90	Tail sheath protein of bacteriophage T4	Phage protein [*Bartonella vinsonii*]/ WP_010704780.1/0.0
Hypothetical protein	YP_034106	41120/372	[pfam13252], Protein of unknown function/1.42e-139	Bacterial and viral proteins with uncharacterized function	Phage related protein [*Bartonella grahamii as4aup*]/ YP_002972501.1/0.0
Phage protein gp27	CAF27127	42012/368	cl01817, Tail_P2_I, Phage tail protein (Tail_P2_I)/3.74e-07	Phage tail protein	Phage protein [*Bartonella tribocorum* CIP 105476]/ YP_001608790.1/0.0
Major tail sheath protein FI	YP_033154	44358/440	cl01389, Phage_sheath_1, Phage tail sheath protein/4e-94	Phage tail sheath protein	Phage-related tail sheath protein [*Bartonella tribocorum* CIP 105476/ YP_001608782.1/0.0
Hypothetical prophage protein	CAF27125	45034/368	[cl15092], Phage tail repeat like/ 2.23e-04	Probably structural protein of phage tail fibre baseplate, potential function in bacterial pathogenicity or interactions	Phage tail collar protein [*Bartonella grahamii as4aup*]/ YP_002971363/ 6e-117
Phage protein gp17	CAF27144	59214/518	[pfam05136], Phage portal protein, lambda family/ 2.54e-83	Protein forming portal, which enables DNA passage during packaging and ejection.	Phage portal protein [*Bartonella birtlesii*]/ WP_017196268.1/0.0
Phage terminase large subunit (gp15)	CAF27146	72486/642	[pfam05876], Phage terminase large subunit (GpA)/00	Site-specific binding and cutting of the DNA in the initial stages of packaging	Terminase large subunit [*Bartonella tribocorum* CIP 105476]/ YP_001608808/0.0

Phage tail protein	CAF27117	82127/766	TIGR01760, tape_meas_TP901, phage tail tape measure protein, TP901 family, core region/ 9.36e-06	Phage tail assembly and tail length determinant	Phage tail protein [*Bartonella grahamii as4aup*]/ YP_002971845/0.0

aa – aminoacids
- unknown

Table 2. The List of proteins identified by LC-MS/MS analysis of bacteriophage preparations from *B. grahamii*. The proteins identified by minimum two peptide matches and with statistically significant Mascot scores above threshold value indicating 95% correctly assigned peptides (p≤0.05) were included. Minimum Mascot score value of matches used in this study was 68.

Protein name	NCBI Accession Number	Protein mass/aa	Motifs/Expect	Function	Other homologs/Accession number
Single-strand binding protein	YP_002972166	18827/168	COG0629, Ssb, Single-stranded DNA-binding protein/ 5e-30	DNA replication, recombination, and repair	Single-stranded DNA-binding protein [*Bartonella elizabethae*]/ WP_005773335.1/6e-114
Hemin binding protein c	YP_002971304	29756/277	COG3637, Opacity protein and related surface antigens/ 2.66e-22	Cell envelope biogenesis, outer membrane	Hemin binding protein C [*Bartonella tribocorum* CIP 105476]/ YP_001608744.1/0.0
Hemin binding protein d	YP_002971524	30074/272	[COG3637], Opacity protein and related surface antigens/ 8.22e-25	Cell envelope biogenesis, outer membrane, Bacterial surface antigens expressed on the surface of pathogens	Hemin binding protein D [*Bartonella tribocorum* CIP 105476]/ YP_001609129.1/ 3e-157
Hemin binding protein a	YP_002971305	30709/288	[COG3637], Opacity protein and related surface antigens / 8.10e-26	Cell envelope biogenesis, outer membrane	Hemin binding protein a [*Bartonella henselae str.* Houston-1]/ YP_033108/ 9e-131 Pap31 [*Bartonella henselae phage* 60457]/ AAC39274/ 1e-128
Phage protein gp20	YP_002971376	40394/358	[pfam03864], Phage major capsid protein E/ 9.14e-87	Stabilisation of the condensed form of the DNA molecule in phage heads.	Phage protein [*Bartonella sp.* AR 15-3]/ CBI79567.1/0.0
Phage tail sheath protein FI	YP_002971362	49305/463	[cl01389], Phage tail sheath protein/2.43e-83	Phage tail sheath	Tail protein [*Bartonella doshiae*]/ WP_004856878.1/ WP_004856878.1/0.0
Putative phage portal protein	YP_002972503	70840/616	-	-	Phage portal protein [*Bartonella elizabethae*]/ WP_005775294.1/0.0

aa - aminoacids
- unknown

assisted in description of their functions and in the classification of identified bacteriophages.

The size and morphology of the phages with smaller diameter corresponds to bacteriophage-like particles occurring in *Bartonella* culture supernatants [9,10]. Nevertheless, the existence of bacteriophages with diameter > 40 nm was reported by Barbian and Minnick (2000) [11], who detected round to icosahedral phages with diameter 80 nm in *B. baciliformis* culture. But according to their transmission electron micrographs, particle sizes were rather heterogeneous in their sizes with diameters 40-80 nm. Likewise, the presence of bacteriophages with diameter > 40 nm was confirmed in BG [17] with head size in the range 50-70 nm.

Tailed phages are the most efficient gene-transfer particles developed in the process of evolution. Previous observations performed using *Bartonella* cultures suggested the presence of tailed bacteriophages. Umemori et al. (1992) [9] reported the presence of tailed bacteriophages with diameter 40 nm and short tail with length 16 nm. Furthermore, Berglund et al. (2009) [17] observed the presence of sheathed tails approximately 100 nm long in BG. Our proteomic analysis confirmed the presence of tail proteins with homology to viruses of family *Myoviridae* in both bacterial cultures. Major tail sheath protein FI identified in BH and BG bacteriophage preparations is occurring in T4-like viruses and bacteriophages P2 (Table 1 and 2). Likewise, structural protein GP20 with homology to T4 bacteriophage capsid [18] and major structural unit of contractile P2 bacteriophages phage tail tube protein FII were identified in BH culture (Table 1). Among other tail proteins confirming presence of P2-like phages were P2-like phage tail protein, P2 GPu protein functioning in tail assembly of λ-like phages and phage tail measure protein participating on phage tail assembly and determining length of phage tail. Another protein identified in BG bacteriophage preparation is putative phage portal protein (Access. No. YP_002972503) with partial

similarity to protein gp1, which is forming bacteriophage portal vertex structure of bacteriophage P22. It has a function during membrane binding and penetration of host membrane as well as a plug holding phage DNA inside of capsid [19]. Single-strand binding proteins functioning in protection of ssDNA intermediates during DNA metabolism, were identified in both BG and BH bacteriophage preparations. Further large subunit of DNA packaging enzyme (gp15), several anti-repressor proteins and phage-related proteins have been found as well. Finally, another component of phage tail (gp27), baseplate (gp26, gp25), tail sheath (gp18), portal protein (gp17) and neck protein (gp13) were identified.

Bacteriophages with similar morphology to the bacteriophage-like particles and the large enveloped phages described in the current study were found in proteobacterium *Pseudomonas* sp. This phytopathogenic bacterium is the host of two different bacteriophages, a dsDNA bacteriophage Psp231a belonging to the family *Podoviridae* (*P. phaseicola*, [20]) and an enveloped dsRNA bacteriophage Φ6-Φ12 [21,22]. The bacteriophage-like particles (diameter 42 nm) are comparable to icosahedral phages Psp231a with diameter size 55 nm and with easily separable tails. The structure of another *Cystoviridae* bacteriophage Φ12 described by Wei et al (2009) which is surrounded by a lipid-containing envelope required for infectivity reminds the enveloped phage particles in our *Bartonella* preparations, though *Bartonella* bacteriophage sizes are smaller than in family *Cystoviridae*. This phage is able to infect not only *Pseudomonas* sp., however also other Gram-negative bacteria.

One of the most interesting proteins found in BH bacteriophage preparations is phage protein CAF27122. The protein displays significant similarity to the phage protein gp79 having inhibitory effect on host transcription [23] or having cytotoxic effect on bacterial host [24]. These characters might be useful for development of phage therapy or study of potential drug targets against bacterial pathogens.

Functions of hemin-binding proteins and Pap31, a protein with high homology to hemin-binding protein in BH (99 % identity) has not been fully elucidated yet. However, common feature of these cell surface proteins is that they are conserved in several plant and animal pathogens within α-proteobacteria [25]. Pap31 was originally considered as a phage-associated membrane protein in BH [10,26]. But later it was suggested, that this protein is a bacteriophage receptor on the outer surface of the bacterium serving for bacterial iron acquisition [27]. Heme acquisition by Pap31 makes this protein potentially important virulence factor in the pathogenesis of BH [28]. Membranes of enveloped phages-such as Φ12, fuse during the first stage of infection with bacterial outer membrane. Therefore during the process called targeted protein-dependent fusion, phage phospholipids and membrane proteins might co-purify with the bacterial outer membrane of infected cells [29].

Conclusions

Present study has shown the occurrence of bacteriophages from different families in human-pathogenic bacteria *B. henselae* and *B. grahamii*. Most of the results of our observations suggests, that *B. henselae* is a host of tailed dsDNA bacteriophages belonging to order *Caudovirales* and family *Myoviridae* (similar to bacteriophage P2), and enveloped bacteriophages similar to dsRNA viruses from family *Cystoviridae*. The Small-size bacteriophage-like particles could correspond to defective or satellite phages. Similar conclusions could be drawn for *B. grahamii*, though less experimental evidences are available.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

JN performed experimental work including bacterial culture, bacteriophage isolation, SDS-PAGE and transmission electron microscopy, experimental planning and manuscript writing. MLN conducted proteomic analysis using LC-MS/MS and contributed to the manuscript writing by method description. All authors read and approved manuscript.

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