Virus Genes (2010) 41:450–458
DOI 10.1007/s11262-010-0525-0

Genome sequence of the temperate bacteriophage PH10 from *Streptococcus oralis*

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Received: 2 June 2010 / Accepted: 17 August 2010 / Published online: 28 August 2010

Abstract Exponential growing cultures of *Streptococcus oralis* strain OMZ 1038, isolated from human supragingival dental plaque, were found to release a bacteriophage (designated PH10) upon treatment with mitomycin C. The complete genome sequence of phage PH10 was determined. The genome was 31276 bp in size and contained 54 open reading frames. The module encoding structural proteins was highly similar to that of *Streptococcus pneumoniae* prophage PhiSpn_3. The most abundant phage structural protein was encoded by ORF35 and was likely processed by proteolytic cleavage. The putative endolysin from PH10, which contained a muramidase domain and a choline-binding domain, was purified and shown to have lytic activity with *S. oralis*, *S. pneumoniae* and *Streptococcus mitis*, but not with other streptococcal species.

Keywords *Streptococcus oralis* · Endolysin · Bacteriophage · Integrase · Attachment site · Prophage

Introduction

The human oral cavity is inhabited by more than 10^{10} bacteria that belong to the level of the individual to more than 100 and at the population level to at least 500 different species [1]. However, only a few of these species may cause disease. One of the largest groups of bacteria found in the oral cavity is the streptococci, accounting for 25–50% of the oral microbiota. They are Gram-positive facultative anaerobes and belong to the order *Lactobacillales*, which harbours bacteria that produce lactic acid from the fermentation of sugars. Based on genetic and phenotypic traits, the streptococci can be subdivided into several major groups, of which the anginosus group, the mitis group and the mutans group are particularly prominent in the human oral cavity.

One of the most important human pathogens, *Streptococcus pneumoniae*, is a member of the mitis group, but it is not a frequent inhabitant of the oral cavity, in contrast to *Streptococcus gordonii*, *Streptococcus mitis*, *Streptococcus oralis* and *Streptococcus sanguinis*. *S. mitis* and *S. oralis* are amongst the first to colonize the oral cavity of neonates [2] and considered commensals, which might play a role in preventing colonization by other pathogenic organisms [3]. All members of the mitis group have been associated with endocarditis though [4]. Most mitis streptococci are naturally competent for genetic transformation [5] and the close genetic relationship between mitis streptococci, especially between *S. pneumoniae*, *S. oralis* and *S. mitis*, is probably at least in part caused by homologous recombination following transformation. For example, it has been suggested that mutations that lead to penicillin resistance in *S. pneumoniae* have been acquired from *S. oralis* or *S. mitis* [6].

Apart from horizontal gene transfer by transformation, evolution of the mitis streptococci may also have been shaped by bacteriophages. Although a wealth of information exists on the bacteria thriving in the oral cavity, relatively little is known about the occurrence of bacteriophages in this environment and their impact on its ecology. Attempts to isolate lytic bacteriophages from human saliva or dental plaque have been successful for

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some host species, for example for *Actinomyces naeslundii* [7] and for *Streptococcus mutans* [8], but others have reported difficulties in isolating bacteriophages [9, 10]. The presence of functional prophages in oral microorganisms has been experimentally demonstrated for *Aggregatibacter actinomycetemcomitans* [11], *S. mitis* [12], *S. gordonii* [13] and *Treponoma denticola* [14].

About half of the clinical *S. pneumoniae* isolates have been found to harbour prophages [15]. Whereas a relatively large amount of information is available with regard to *S. pneumoniae* and *S. mitis* bacteriophages [12, 16, 17], little is known about bacteriophages from *S. oralis*. Interestingly, some virulent *S. pneumoniae* bacteriophages were capable of replicating in *S. oralis* [18], highlighting the close relationship between these species. I have previously reported about the presence of prophages in three oral streptococcal strains isolated from human supragingival plaque and about the characterization of bacteriophage PH15, which resides as prophage in the genome of *S. gordonii* OMZ 1039 [13]. This study deals with prophage PH10 from *S. oralis* strain OMZ 1038.

Materials and methods

Bacterial strains and growth conditions

The streptococcal strains used in this study were from the laboratory collection and are listed in Table 1. Streptococci were routinely grown in Todd-Hewitt broth supplemented with 0.3% yeast extract (THY) at 37°C. *Escherichia coli* strains JM109 and BL21(DE3) (Novagen) were used as host for propagation of plasmids and for protein expression, respectively, and grown in LB medium. When required, kanamycin was added to a final concentration of 50 μg/ml.

Preparation of phage lysates and electron microscopy

For preparation of phage PH10, overnight cultures of *S. oralis* OMZ 1038 were 100-fold diluted in fresh THY medium. After 3 h of incubation at 37°C, mitomycin C was added to a final concentration of 0.1 μg/ml. After incubation for another 20 h at 37°C, the lysate was centrifuged for

Table 1 Activity of purified endolysin from PH10 with streptococci

Organism	Strain	Original designation, characteristics	Lysis by PH10 endolysin
Streptococcus oralis	OMZ 1038	Harbours prophage PH10	+
Streptococcus oralis	OMZ 1081	Harbours prophage PH5	+
Streptococcus oralis	OMZ 8	ATCC 9811	+
Streptococcus oralis	OMZ 507	SK2 (NCTC 7864)	+
Streptococcus oralis	OMZ 607	SK248	+
Streptococcus mitis	OMZ 508	SK24 (NCTC 8029)	+
Streptococcus mitis	OMZ 991	DSMZ 12643 (NCTC 12261)	+
Streptococcus mitis	OMZ 1049	BA	+
Streptococcus pneumoniae	OMZ 810	Wu2	+
Streptococcus gordonii	OMZ 1039	Harbours prophage PH15	−
Streptococcus gordonii	OMZ 504	ATCC 12396 (SK6)	−
Streptococcus gordonii	OMZ 938	Challis DL1	−
Streptococcus sanguinis	OMZ 499	ATCC 10556	−
Streptococcus sanguinis	OMZ 500	SK36	−
Streptococcus sanguinis	OMZ 503	SK46	−
Streptococcus mutans	OMZ 381	P42	−
Streptococcus mutans	OMZ 918	UA159 (ATCC 700610)	−
Streptococcus anginosus	OMZ 510	NCTC 10713	−
Streptococcus anginosus	OMZ 587		−
Streptococcus constellatus	OMZ 511	ATCC 27823	−
Streptococcus intermedius	OMZ 512	ATCC 27335	−
Streptococcus salivarius	OMZ 513	NCTC 8618	−
Streptococcus salivarius	OMZ 867	NCTC 8606	−
Streptococcus agalactiae	OMZ 1093		−
Streptococcus pyogenes	OMZ 1092		−
Enterococcus faecalis	OMZ 940	GC110	−

20 min. at 5,000*g*. Subsequently, the supernatant was centrifuged for 1 h at 4°C at 100,000*g*. The pellet was dissolved in 0.1 M NH_4Ac pH 6.7. Electron microscopy was carried out with a Philips EM 400T electron microscope at 60 kV and using phosphotungstic acid as negative stain as described before [13].

DNA manipulations

Plasmid DNA purification from *E. coli*, restriction enzyme digestion, agarose gel electrophoresis, DNA ligation and transformation of *E. coli* were carried out using standard methods [19]. Chromosomal DNA was isolated by using the Genelute Bacterial genomic DNA kit (Sigma). DNA sequencing employed dye-terminators and was performed on ABI 3730xl (Applied Biosystems).

Isolation of phage DNA, sequencing strategy and sequence analysis

For isolation of phage DNA, a lysate from *S. oralis* OMZ 1038 prepared as described above was centrifuged for 10 min at 7,000*g* and the supernatant passed through a 0.4-μm membrane filter. Phage DNA was isolated from the lysate by using the Lambdaprep kit (Promega). *Eco*RV-digested phage DNA was cloned into the vector pCC1 (Epicentre), which yielded 14 clones with different inserts with a total size of about 26 kb. These inserts were sequenced in both directions by a primer-walking strategy. Direct sequencing in both directions of PCR fragments obtained with custom primers was utilized to confirm junction sequences across the ends of cloned fragments or to fill gaps. Sequences were assembled with the program CAP3 [20]. The nucleotide sequence reported here is deposited in the EMBL database under accession number FN391954 and in the NCBI genome database under NC_012756.

The assembled sequence of PH10 was analysed for the presence of open reading frames with the program Genemark.hmm [21] using the codon usage of *Streptococcus pneumoniae* strain R6. Further sequence analysis used the programs from the GCG package (Accelrys, Cambridge, UK). Protein sequences were compared with the non-redundant protein sequence database using BlastP at http://www.ncbi.nlm.nih.gov/blast/, which includes a search for conserved domains.

Identification of the major capsid protein

The structural proteins of PH10 were separated by subjecting a preparation of bacteriophage PH10 to 10% SDS-PAGE. After electrophoresis, the gel was stained with Coomassie brilliant blue and the most intense band, corresponding to a size of about 35 kDa, was cut from the gel. The gel band was cut in small pieces, washed twice with 100 μl of 100 mM NH_4HCO_3/50% acetonitrile, and washed once with 50 μl acetonitrile. 10 μl of a solution of trypsin (10 ng/μl in 10 mM Tris/2 mM $CaCl_2$, pH 8.2) and 10 μl of buffer (10 mM Tris/2 mM $CaCl_2$, pH 8.2) were added to the pellet. The mixture was incubated overnight at 37°C. After centrifugation, the supernatant was removed and the gel pieces were extracted twice with 100 μl of 0.1%/50% acetonitrile. All three supernatants were combined, dried and dissolved in 25 μl of 0.1% formic acid, and 1 μl was used for LC/MS/MS on a QTOF Ultima API (Waters). The peptide mass fingerprint was scanned against the protein sequences from PH10 by using the Mascot search program.

Cloning, expression and activity of the lytic enzyme of PH10

The *lysA* gene from PH10 was amplified by PCR using primers ph10lysf and ph10lysr and genomic DNA from strain OMZ 1038 as template. The product was digested with *Nde*I and *Xho*I and cloned into similarly digested pET28b (Merck) to give plasmid pOMZ286. Plasmid pOMZ286 was introduced into *E. coli* BL21(DE3) (Merck) by transformation. Expression and purification by Ni–NTA agarose affinity chromatography was as described previously [13]. For mass spectrometry analysis, a sample of the protein was purified on C4 ZipTips (Millipore) and measured in 50% acetonitrile/0.2% formic acid (pH 2) on a Q-TOF Ultima API mass spectrometer. The mass spectrum (average mass of the neutral molecule) was deconvolved using MaxEnt1 software.

Cell wall lytic activity of the purified protein with different bacterial strains was evaluated as described before [13] or by spectrophotometric measurement of the optical density of bacterial cultures, over time, after addition of enzyme.

Results and discussion

Identification of *S. oralis* strain OMZ 1038 and bacteriophage PH10

Strain OMZ 1038 has been previously assigned as *S. oralis*, based on the sequence of *sodA* [13]. Conclusive evidence for the assignment for strain was obtained by sequencing part of the *gdh* and *ddl* genes (data not shown). Electron microscopic analysis of lysates of strain OMZ 1038 after treatment with mitomycin C showed the presence of bacteriophages (designated PH10; Fig. 1), whose structure suggested that it belongs to the *Siphoviridae*. The length of

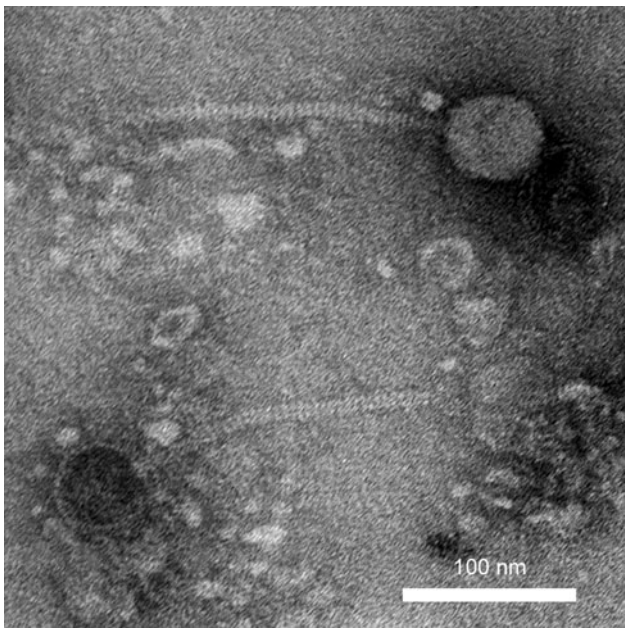

Fig. 1 Electron microscopic image of *S. oralis* bacteriophage PH10

the phage tail was 157.5 ± 11.7 nm ($n = 37$), whereas the diameter of the phage head was 53.9 ± 2.6 nm ($n = 35$).

Genome sequence of phage PH10

The complete genome sequence of PH10 was determined to be 31276 bp in size. The GC percentage of the genome was 39.5%, which is somewhat lower than the reported GC percentages of *S. oralis* genomes (41–42%) [22]. A total of 54 ORFs were encoded on the phage genome (Table 2; Fig. 2). Six of the ORFs had a TTG start codon (ORF13, ORF23, ORF27, ORF31, ORF46 and ORF54) whereas the remaining ORFs had an ATG start codon. The transcription of most ORFs was directed to the right, but eight ORFs, all located proximal to the phage attachment sites, were oriented in the opposite direction (Fig. 2). Based on sequence similarity, the ORFs were assigned to different functional groups, e.g. lysogeny, replication and regulation, structure, lysis and lysogenic conversion. Bacteriophages from low GC content Gram-positive organisms have a conserved genetic organization [23], which is also present in bacteriophage PH10. Overall, the genome sequence showed most similarity to the prophage phiSpn_3 from *S. pneumoniae* strain CSGSSp3BS71 (Fig. 2) [15, 24].

Lysogeny module

The lysogeny module is located on the leftward part of the genome (Fig. 2). The overall organization of the lysogeny module was similar to those found in phages from other low GC Gram-positive bacteria [25]. The first ORF is predicted to encode an integrase. It showed high sequence similarity (96% identity) with the putative integrase from *S. mitis* bacteriophage SK137 [26] and with putative phage integrases deduced from genomic sequences of *S. pneumoniae* strains. Using PCR analysis, this type of phage integrase has recently been shown to be present in more than half of lysogenic pneumococci [15].

The deduced protein sequence encoded by ORF4 contains a helix-turn-helix DNA binding domain, suggesting that it encodes the phage repressor. The intergenic region upstream of the phage repressor usually harbours the genetic switch for lysogeny/lysis. However, possible binding sites for the phage repressor could not be identified.

Replication and regulation module

Most ORFs of this module showed similarity to ORFs of unknown function from streptococcal bacteriophages. ORF11 possibly encodes the initiator of DNA replication, since it contains a phage replisome organiser domain and a DnaD domain. Because a gene encoding a putative helicase appears to be absent, the replication module of PH10 may belong to the initiator-solo type of replication modules found in several phages of Gram-positives [27]. The origins of replication of a number of lactococcal bacteriophages are located in the central part of the gene encoding the replisome organizer and include a region with direct repeats [28]. A 16 bp direct repeat separated by 9 bp was also found in the central part of ORF11 from PH10 (not shown), indicating that it could function as origin of replication. It cannot be excluded, however, that the repeats serve a structural function of the encoded protein.

Of interest is the similarity of ORF13 and ORF14 to putative C-5 cytosine-specific DNA methylases from *S. mitis* bacteriophage VO1 (accession number CAD35756) [29] and *Neisseria gonorrhoeae* MS11 (accession number AJ004687) [30]. It appears that in PH10, a frame shift mutation in a homopolymeric G-tract resulted in a premature stop in ORF13. This mutation could have been introduced after cloning of the phage DNA fragment in *E. coli*, but resequencing of the product obtained after PCR amplification from genomic DNA of strain OMZ 1038 confirmed that the sequence was correct (data not shown). Interestingly, it has been demonstrated that the protein encoded by *N gonorrhoeae* MS11 is inactive. It was hypothesized that this was caused by a frame shift [31]. Both frame shift mutations are located in a homopolymeric G-tract which contains 10 G residues in the PH10 sequence and 9 in the *N. gonorrhoeae* MS11 sequence, whereas bacteriophage VO1 sequence has 8 G residues. The observation that the frame shift mutations were present in homopolymeric tracts, suggests that they may be regulated

Table 2 ORFs encoded by bacteriophage PH10

ORF[a]	From bp	To bp	aa	Identity over aa	% identity	To [organism] accession number	Conserved domain	(Putative) function
1	170	1318	382	382	96	Integrase [Phage SK137] CAJ13674	INT-P4; Int-PhilC3-3	Integrase
2	1493	1900	135	135	86	Hypothetical protein [*S. agalactiae* 2603 V/R] AAN00746		
3	1925	2326	133	126	62	Hypothetical protein SAG1883 [*S. agalactiae* 2603 V/R] AAN00745	DUF955	
4	2337	2684	115	117	66	Phage transcriptional regulator, Cro/CI family protein [*S. pneumoniae* SP9-BS68] EDK78989	HTH-XRE	CI repressor
5	2989	3198	69	69	53	Hypothetical protein SpyM3_0686 [*S. pyogenes* MGAS315] NP_664490	VapI	
6	3202	3432	76	76	81	gp12 [*S. mitis* phage SM1] NP_862851		
7	3444	3653	69					
8	3713	4426	237	237	97	Prophage LambdaSa2, antirepressor protein, putative [*S. pneumoniae* SP11-BS70] EDK62225	COG3617	Antirepressor
9	4439	4696	85	85	89	Hypothetical protein CGSSp11BS70_06605 [*S. pneumoniae* SP11-BS70] EDK62226		
10	4776	4988	70	72	63	Hypothetical protein [*S. agalactiae* 2603 V/R] NP_688863		
11	5009	5881	290	288	93	gp49 homologous [*N. gonorrhoeae*] CAC28357	Phage_rep_org_N; DnaD	Replication protein
12	5881	6075	64	63	73	Hypothetical protein CGSSp11BS70_06635 [*S. pneumoniae* SP11-BS70] EDK62232		
13*	6076	6216	46	33	69	C-5 cytosine-specific DNA methylase [*S. pneumoniae* SP18-BS74] EDK67432		Methylase
14	6207	6875	222	221	72	C-5 cytosine-specific DNA methylase [*S. pneumoniae* SP18-BS74] EDK67432		Methylase
15	6868	7089	73	67	50	Hypothetical protein CGSSp3BS71_04124 [*S. pneumoniae* SP3-BS71] EDK72974		
16	7091	7582	163	141	97	Hypothetical protein [*N. gonorrhoeae*]. CAC28358		
17	7584	7850	88					
18	7865	8014	49					
19	8011	8337	108	84	105	Hypothetical protein CGSSp9BS68_09362 [*S. pneumoniae* SP9-BS68] EDK79017		
20	8327	8743	138	138	80	Prophage LambdaSa2, single-strand binding protein [*S. agalactiae* 2603 V/R] NP_688853	SBB_OBF	Single stranded binding protein
21	8757	9092	111	109	34	Hypothetical protein -phage-associated [*S. pyogenes* MGAS315] AAM79940		
22	9105	9491	128					
23*	9488	9736	82	86	40	gp27 [*S. mitis* phage SM1] AAP81909	DUF1372	
24	9733	10134	133	132	84	Hypothetical protein [Bacteriophage EJ-1] CAE82112		
25	10164	10415	83	68	63	Hypothetical protein SAG1862 [*S. agalactiae* 2603 V/R] NP_688852		
26	10406	10744	112	108	59	Prophage LambdaSa2, transcriptional regulator, Cro/CI family [*S. agalactiae* 2603 V/R] NP_688851	HTH-XRE	Immunity repressor
27*	10741	11142	133	133	94	Putative transcriptional activator (phage 31 late promoter) [*S. pneumoniae* SP3-BS71] EDK72981		
28	11334	11876	180	180	100	Prophage LambdaSa2, site-specific recombinase, phage integrase family protein [*S. pneumoniae* SP3-BS71] EDK72982	INT_SG3_C	
29	12040	12258	72					

Table 2 continued

ORF[a]	From bp	To bp	aa	Identity over aa	% identity	To [organism] accession number	Conserved domain	(Putative) function
30	12389	12715	108	102	86	Hypothetical protein CGSSp3BS71_04169 [*S. pneumoniae* SP3-BS71] EDK72983		Endonuclease
31*	12826	13218	130	130	96	Phage-related terminase protein small subunit, putative [*S. pneumoniae* SP3-BS71] EDK72984		Terminase small subunit
32	13211	14941	576	576	95	Terminase [*S. pneumoniae* SP3-BS71] EDK72985	DEX_DC; COG4626	Large terminase
33	14949	15167	72	72	91	Hypothetical protein CGSSp3BS71_04184 [*S. pneumoniae* SP3-BS71] EDK72986		
34	15185	16387	400	400	71	Phage portal protein, putative [*S. pneumoniae* SP3-BS71] EDK72987	Phage_Portal	Portal protein
35	16371	16961	196	192	85	Phage maturation protease, putative [*S. pneumoniae* SP3-BS71] EDK72988	COG3740	
36	16946	18118	390	390	52	Capsid protein [*S. pneumoniae* SP3-BS71] EDK72989	Phage_Capsid	Phage capsid
37	18130	18432	100	74	51	Hypothetical protein CGSSp3BS71_04204 [*S. pneumoniae* SP3-BS71] EDK72990		
38	18435	18716	93	93	74	Prophage pi2 protein 35 [*S. pneumoniae* SP3-BS71] EDK72991		
39	18703	19002	99	99	93	Phage-related protein, putative [*S. pneumoniae* SP3-BS71] EDK72992		Phage head–tail adaptor
40	18999	19346	115	114	42	Hypothetical protein CGSSp3BS71_04219 [*S. pneumoniae* SP3-BS71] EDK72993		
41	19343	19666	107	107	90	Prophage pi2 protein 38 [*S. pneumoniae* SP3-BS71] EDK72994		
42	19678	20256	192	192	79	Prophage pi2 protein 39 [*S. pneumoniae* SP3-BS71] EDK72995		
43	20268	20687	139	139	94	Hypothetical protein CGSSp3BS71_04234 [*S. pneumoniae* SP3-BS71] EDK72996		
44	20965	23979	1004	1037	61	Tail tape measure protein [*S. agalactiae* CJB111] EAO73646	COG5280, COG5412	Tail tape measure
45	23973	24695	240	240	94	Hypothetical protein CGSSp3BS71_04249 [*S. pneumoniae* SP3-BS71] EDK72999		
46*	24696	28109	1137	531	81	Prophage LambdaSa2, PblB, putative [*S. pneumoniae* SP3-BS71] EDK73000	PblB	
47	28090	28293	67	67	94	Hypothetical protein CGSSp6BS73_12621 [*S. pneumoniae* SP6-BS73] EDK72938		
48	28296	28931	211	211	57	unknown [*S. mitis* phage SM1] AAK37662		
49	28941	29222	93	91	76	Hypothetical protein EJ-1p68 [*Streptococcus* phage EJ-1] CAE82150		
50	29237	29515	92	67	94	Hypothetical protein CGSSp9BS68_00732 [*S. pneumoniae* SP9-BS68] EDK77849		Holin
51	29525	30529	334	338	69	Lysozyme [*Streptococcus* phage Cp-1] CAA87744	Glyco_25; COG5263	Endolysin
52	30811	30632	59	59	94	Hypothetical protein CGSSp11BS70_06990 [*S. pneumoniae* SP11-BS70] EDK64035		
53	30887	30813	24	24	95	Hypothetical protein CGSSp11BS70_06995 [*S. pneumoniae* SP11-BS70] EDK64036		
54*	31102	30953	49	37	100	Hypothetical protein CGSSp11BS70_07000 [*S. pneumoniae* SP11-BS70] EDK64037		

[a] ORFs with a TTG start codon are indicated by an asterisk

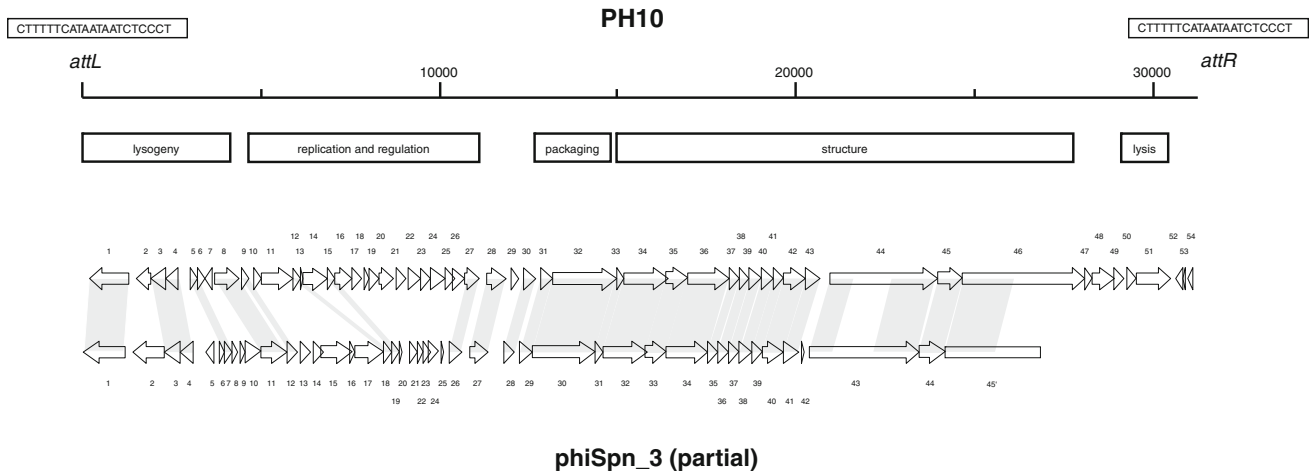

Fig. 2 Genomic organization of *S. oralis* bacteriophage PH10 and comparison with *S. pneumoniae* prophage phiSpn_3. Different modules are indicated. ORFs with sequence similarity to ORFs (greater than 30% identity) from phiSpn_3 are indicated by *grey shading* between both genomes

by phase variation through slipped-strand mispairing during DNA replication [32].

The sequence similarity of PH10 with the fragment from *N. gonorrhoeae* MS11 was not confined to the DNA methylase. Alignment of both sequences showed an overall nucleotide sequence identity of about 90% over 3.8 kb with two gaps in the PH10 sequence. The GC content of the sequence from *N. gonorrhoeae* MS11 is 38%, whereas the GC content of another strain of *N. gonorrhoeae* (NCCP11945) has been reported to be 52.4% [33]. It seems, therefore, likely that this fragment from *N. gonorrhoeae* MS11 has originated from a streptococcal bacteriophage or from some other low GC Gram-positive bacteriophage.

Structural module

The structural module (ORF34–ORF46) of PH10 exhibited the order portal protein, major capsid protein, major tail protein, tail tape measure protein. This order is conserved in several bacteriophages from low GC content bacteria [12]. All ORFs encoded by the structural module showed high sequence similarity and conservation of synteny with ORFs from prophage phiSpn_3 from *S. pneumoniae* strain CSGSSp3BS71, which was isolated from the nasopharynx (Fig. 2) [15, 24]. The similarity with phiSpn_3 ended in ORF46, since the contig from the genome sequence of CSGSSp3BS71 finished here. Except for ORF46, which showed 31% identity to PblB, there was no similarity of the PH10 structural ORFs with the ORFs from the structural module of *S. mitis* bacteriophage SM1, which is also a member of the *Siphoviridae* [12].

Upon separation of the bacteriophage structural proteins by SDS-PAGE, a major band with a size of about 35 kDa was observed. Using mass spectrometry, the protein representing this band was identified as ORF36 (not shown). Since the calculated mass of ORF36 is 43.6 kDa, it is likely that the protein had been processed by proteolytic cleavage. This is supported by the observation that peptides representing the first 126 amino acids were not found in the peptide mass fingerprint. In addition, ORF36 is preceded by a putative phagehead protease from the HK97 family (Table 2).

Lysis module

ORF50 exhibited sequence similarity to putative holins from *S. mitis* bacteriophage Sk137, *S. pneumoniae* bacteriophage EJ-1 and *S. gordonii* bacteriophage PH15. ORF51 contains a muramidase domain and a choline-binding domain and was highly similar (69% identity) to endolysin Cpl-1 from *S. pneumoniae* phage Cp-1, which has been extensively characterized [34] and whose three-dimensional structure has been determined [35, 36].

Region downstream of lysis module

The region downstream of the lysis module and flanking the *attR* site frequently encodes lysogenic conversion genes [23]. In PH10, this region contains 3 ORFs (ORF52 to ORF54), which are transcribed in the direction opposite to that of ORF51. The amino acid sequences encoded by these ORFs did not show sequence similarity to those of proteins of known function.

Phage attachment site in OMZ 1038

The phage and bacterial attachment sites (*attP* and *attB*) were determined by direct sequencing of genomic DNA as described previously [13], using primers derived from the sequence of PH10. The phage attachment site had the following sequence: 5′-CTTTTTCATAATAATCTCCCT-3′.

An identical sequence was found in the intergenic region between the genes encoding the endolysin and integrase of *S. mitis* bacteriophage SK137 [26], which strongly suggests that the phage attachments sites of PH10 and SK137 are identical. This hypothesis is supported by the high similarity of the putative bacteriophage integrases (96%).

The attachment site sequence was also found in the genomes of several *S. pneumoniae* strains, i.e. strains SP195 (accession number NZ_ABGE00000000), CDC1873-00 (NZ_ABFS00000000), Hungary19A-6 (NC_010380), SP14-BS69 (NZ_ABAD00000000) and SP11-BS70 (NZ_ABAC00000000). In all these genomes, the attachment site was followed by an ORF which might encode an integrase, the amino acid sequence of which showed more than 90% identity with the putative PH10 integrase.

Functional analysis of the PH10 endolysin

To characterize the PH10 endolysin, the *lysA* gene (ORF51) was cloned into the *E. coli* expression vector pET28b as fusion to a C-terminal His-tag. Upon induction with IPTG, SDS-PAGE analysis showed that a protein with a size of about 35 kDa was produced, which is somewhat lower than the calculated size of the protein (40151.5 Da). Mass spectrometry analysis of the purified protein confirmed that the protein was of the correct size (40151.7 Da). Lytic activity of the protein with different streptococcal species was evaluated. The protein inhibited the growth of all the 9 tested strains of *S. oralis*, *S. mitis* and S. *pneumoniae* strains (Table 1; Fig. 3). The PH10 endolysin was not active with any of the other streptococcal species tested. Although speculative, choline-bound cell wall teichoic acid present in *S. oralis*, *S. mitis* and *S. pneumoniae*, but absent in most other streptococcal species, might function as receptor for the choline-binding domain of LysA. Interestingly, the substrate range of the PH10 endolysin was complementary to that of the endolysin from *S. gordonii* bacteriophage PH15 [13]. This may be of potential interest for application of endolysins to treat endocarditis.

Conclusions

The relative ease with which temperate bacteriophages of mitis streptococci can be isolated suggests that phages for this group of bacteria are widespread. The close relatedness of the *S. oralis* bacteriophage PH10 genome to *S. pneumoniae* bacteriophages phiSpn_3 is in support of the previous observation that some pneumococcal bacteriophages can replicate in *S. oralis* [18]. It is likely that, besides homologous recombination between DNA from residingprophages and infecting phages, homologous recombination following the transformation of naturally competent cells plays a role in evolution of bacteriophages from this group of streptococci. The endolysin encoded by PH10 was only active with the members of this group, most likely due to the requirement for choline-bound teichoic acid, which serves as receptor for the choline-binding domain.

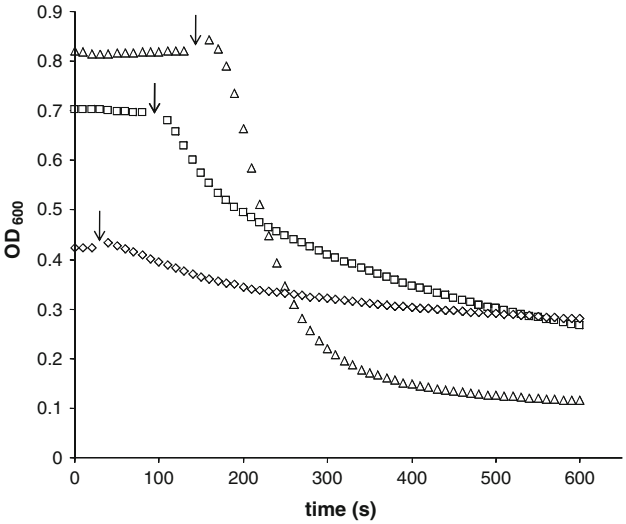

Fig. 3 Lysis of target bacteria by purified endolysin from PH10. Overnight cultures of *S. oralis* OMZ 607 (*squares*), *S. pneumonia* OMZ 810 (*triangles*) and *S. oralis* OMZ 1038 (*diamonds*) were diluted 50-fold into fresh medium and grown for 6 h at 37°C. Cells were washed, re-suspended in PBS and the OD_{600} of 1 ml of cell suspension was monitored on a Hitachi U-2000 spectrophotometer. The arrows indicate the addition of purified enzyme (10 μg)

Acknowledgments The excellent technical assistance of Verena Osterwalder and Steve Reese is gratefully acknowledged. I thank Peter Brunisholz and Serge Chesnov for mass spectrometry analysis.

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