Complete Genomic Nucleotide Sequence of the Temperate Bacteriophage AaΦ23 of *Actinobacillus actinomycetemcomitans*†

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The entire double-stranded DNA genome of the *Actinobacillus actinomycetemcomitans* bacteriophage AaΦ23 was sequenced. Linear DNA contained in the phage particles is circularly permuted and terminally redundant. Therefore, the physical map of the phage genome is circular. Its size is 43,033 bp with an overall molar G+C content of 42.5 mol%. Sixty-six potential open reading frames (ORFs) were identified, including an ORF resulting from a translational frameshift. A putative function could be assigned to 23 of them. Twenty-three other ORFs share homologies only with hypothetical proteins present in several bacteria or bacteriophages, and 20 ORFs seem to be specific for phage AaΦ23. The organization of the phage genome and several genetic functions share extensive similarities to that of the lambdoid phages. However, AaΦ23 encodes a DNA adenine methylase, and the DNA packaging strategy is more closely related to the P22 system. The attachment sites of AaΦ23 (*attP*) and several *A. actinomycetemcomitans* hosts (*attB*) are 49 bp long.

Actinobacillus actinomycetemcomitans is a capnophilic, non-motile gram-negative bacterium which has been strongly implicated in the etiology of several forms of periodontitis and may play a role in extraoral infection. Several putative virulence factors, including a leukotoxin, have been described previously (13).

Lysogeny is widespread in *A. actinomycetemcomitans*. Lysogenic *A. actinomycetemcomitans* isolates have been isolated from periodontal pockets (11, 15, 30, 33) as well as from periodontally healthy individuals (38). The role of phages in the etiology of periodontal diseases is not yet clear. Specifically, members of the AaΦ23 family of phages have been found in about 40% of the *A. actinomycetemcomitans* isolates (11, 15, 25, 38), and AaΦ23 was shown to transduce antibiotic resistance markers in vitro (39). In several bacterial species, such as *Vibrio cholerae* (17, 36) and *Escherichia coli* (24), phage-encoded genes modulate virulence.

In previous work, it was shown that phages released from several *A. actinomycetemcomitans* lysogens possess an isometric icosahedral head of 65 nm, a contractile tail of 110 nm, and a baseplate with up to four fibers and are genetically related (40). Phage particles contain approximately 45 kb of double-stranded linear DNA that is circularly permuted and terminally redundant for 1.6 kb (37). To further our understanding of the biology of *A. actinomycetemcomitans* bacteriophages, we decided to sequence the entire genome of the bacteriophage

AaΦ23 naturally carried by *A. actinomycetemcomitans* strain ZIB1023 (40).

Four shotgun subclone libraries were prepared from purified phage DNA. After sequencing of 289,652 bp, representing coverage of the AaΦ23 genome 6.7 times, the final consensus sequence obtained with phred/phrap/consed (GCG Wisconsin package; Genetics Computer Group, Accelrys, Inc., Munich, Germany) was circular. For convenience, the phage genome was linearized at the first nucleotide of the *attP* core sequence (Fig. 1). The sequence of the AaΦ23 genome is composed of 43,033 bp. Virtual restriction maps of this sequence are in good agreement with the restriction maps previously obtained (40), suggesting correct assembly of the sequences. The average G+C content of AaΦ23 is 42.5 mol%, which is similar to the 42.7 mol% reported for the *A. actinomycetemcomitans* genome (16). Sixty-six potential genes were predicted by analyses of the AaΦ23 genome sequence with the heuristic approaches of version 2.4 of GeneMark and version 2.0 of GeneMarkHMM (http://opal.biology.gatech.edu/GeneMark/heuristic_hmm2.cgi) and confirmed with a modified version of the GeneMarkS program specifically developed for viral genomes (21). The genome of AaΦ23 is very condensed in terms of coding sequences, as 93.8% are covered by open reading frames (ORFs). No tRNA genes are present. Fifteen stem-loop-like structures that may represent rho-independent transcription termination signals have been identified (Fig. 1). The 66 potential genes and the corresponding protein sequences were compared, by using PSI-BLAST (http://www.ncbi.nlm.nih.gov/BLAST), to the GenBank and Swissprot databases. Characteristics of the gene products and the significant homologies are listed in Table 1, and some are detailed below. Twenty ORFs may be specific for AaΦ23, as no homology to other sequences was found. When analyzing putative gene functions, three distinct regions can be delimited on the genome: (i) integration and immunity, (ii) regulation, DNA replication, DNA modification, and recombination, and (iii) lysis and

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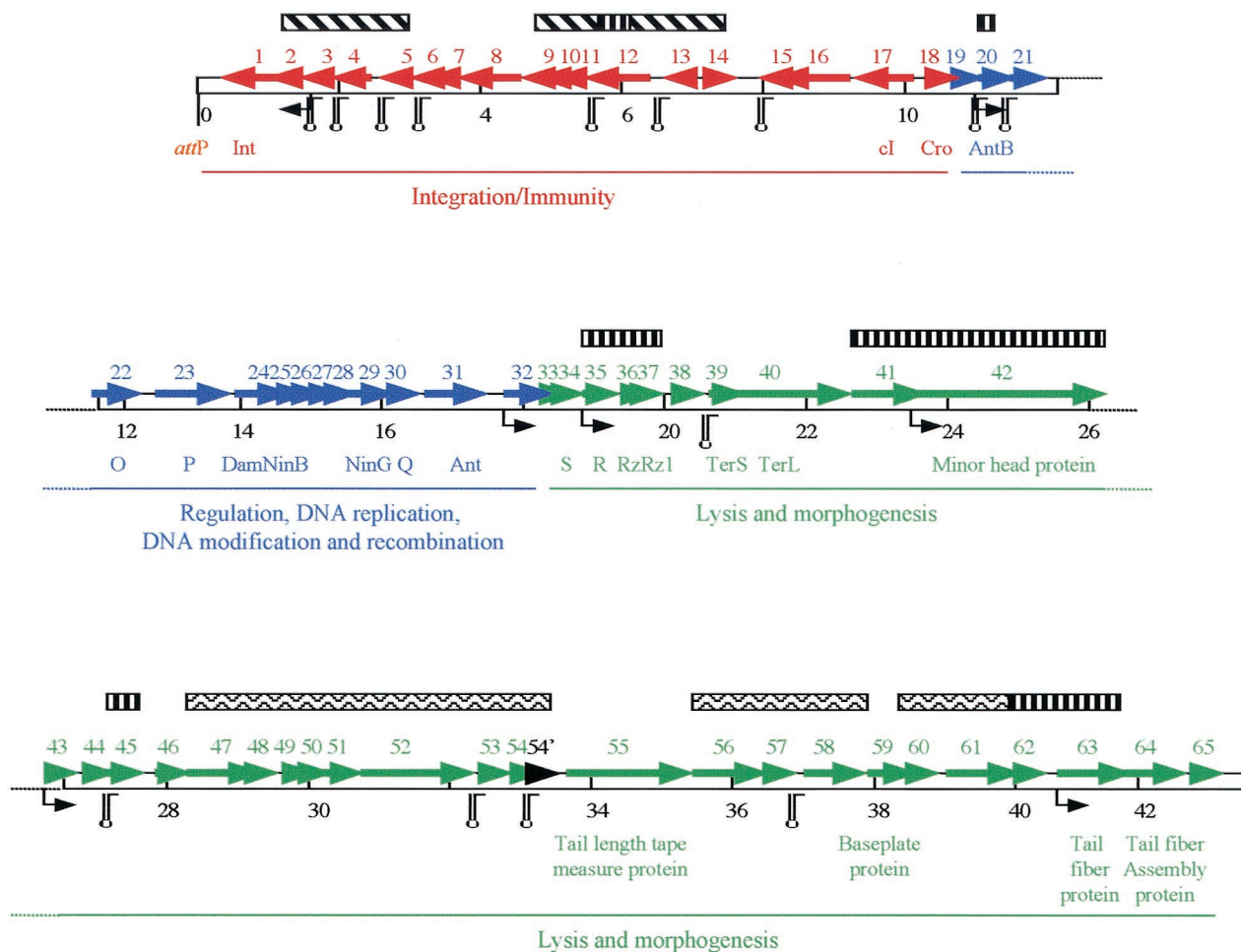
FIG. 1. Schematic representation of the AaΦ23 genome. ORFs are numbered consecutively from left to right and indicated by arrows pointing in the direction of transcription. Putative functions are indicated. Filled boxes with diagonal, vertical, and zigzag lines indicate regions of homology to the genome of *A. actinomycetemcomitans* strain HK1651, the *H. influenzae* ϕflu prophage, and the *X. fastidiosa* Xfp3/Xfp4 prophages, respectively. Rho-independent terminators and promoters are highlighted by hairpins and small black arrows, respectively. The −1 ORF (*orf54′*) is represented by a black arrow.

morphogenesis (Fig. 1). The same genetic organization has also been observed with other bacteriophages such as lambda, P22, L, and LP-7 (5).

ORF1 may represent the integrase of phage AaΦ23 (Table 1 and data not shown). The InterProScan software (http://www.ebi.ac.uk/InterProScan) identified a domain conserved among phage integrases from amino acid 145 to 324 (PFAM accession number PF00589, e value of 1.7e−38). Thus, this protein may be a new member of the large family of Int/recombinases (23) which catalyzes the site-specific integration and excision of the AaΦ23 genome into and out of the host chromosome. As for phage lambda, the putative AaΦ23 integrase gene is located in a cluster of genes transcribed leftwards on the genetic map, i.e., in the opposite orientation to the majority of the phage genes (Fig. 1). No gene coding for an excisionase has been identified on the AaΦ23 genome. This situation is also encountered in other phages, e.g., *Pseudomonas aeruginosa* phage D3 (18). By semirandom PCR, a 49-bp-long direct repeat was identified at the junctions of prophage and bacterial DNA in strain ZIB1023. Moreover, a single copy of this repeat was found in the chromosome of the nonlysogenic strains ZIB1001 and

ZIB1015 and was also evident on the AaΦ23 DNA sequence. When strain ZIB1001 was lysogenized to result in ZIB1515, the prophage was flanked by direct copies of the repeat, representing the *att* site (Fig. 2). Analysis also revealed a single copy of this repeat in the genome of *A. actinomycetemcomitans* strain HK1651 and *Haemophilus influenzae* Rd strain KW20. In AaΦ23, the *attP* site is located 19 bp downstream from the putative integrase gene. The *attB* homologous sequence is located intergenically in the genomes of *A. actinomycetemcomitans* and *H. influenzae*.

ORF17 presents strong homologies to the repressor protein cI of the lambda-like phage phi-80 (9). Moreover, this protein contains a helix-turn-helix motif from amino acid 12 to 66 (PFAM accession number PF013081, e value of 1.1e−08), which is a signature of DNA binding proteins. The product of *orf18* is a 68-amino-acid protein that shares significant homologies with hypothetical proteins and weak homologies to the transcription regulatory protein Cro of several bacteriophages (Table 1 and data not shown). Moreover, three regions of inverted repeats, which may be analogues of the three sequences that compose the O_R operator of phage lambda, and

TABLE 1. Description of bacteriophage AaΦ23 ORFs, gene products, and functional assignments

Gene	Start position	Stop position	Size (aa)[a]	Potential function (gene name)	Significant matches (source, accession no., *e* value)
orf1	1115	69	348	Integrase (*int*)	Integrase (phage P22, P04890, 9*e*−66)
orf2	1336	989	115		
orf3	1666	1352	104		Putative prophage protein (*A. actinomycetemcomitans* strain HK1651, 2*e*−55)
orf4	2377	1721	218		Putative prophage protein (*A. actinomycetemcomitans* strain HK1651, *e*−113)
orf5	2929	2444	161		Putative prophage protein (*A. actinomycetemcomitans* strain HK1651, 6*e*−48)
orf6	3144	2962	60		
orf7	3760	3137	207		
orf8	4650	3757	297		
orf9	4961	4662	99		
orf10	5218	4964	84		
orf11	5483	5208	91		
orf12	6226	5570	218		Putative prophage protein HI1422 (prophage ɸflu, P44193, 8*e*−10)
orf13	6670	6587	83		
orf14	7138	7662	174		Putative prophage protein (*A. actinomycetemcomitans* strain HK1651, 6*e*−78)
orf15	8491	8018	157		
orf16	9347	8460	295		
orf17	10121	9405	250	Repressor (*cl*)	Repressor protein C1 (phage phi-80, P14819, *e*−44)
orf18	10231	10419	68	Repressor (*cro*)	Hypothetical protein YdaS (*E. coli* strain K12, P76063, 7*e*−07); Cro protein (phage P22; P09964, 1.2)
orf19	10410	10736	108		Putative prophage protein (*A. actinomycetemcomitans* strain HK1651, 3*e*−08)
orf20	10788	11507	239	Antirepressor (*antB*)	Putative prophage protein HI1422 (prophage ɸflu, E64029, 6*e*−24); AntB (phage N15, AAM53132.1, *e*−22)
orf21	11507	11665	52		
orf22	11662	12444	260	DNA replication protein (*o*)	Protein GP18 (phage P22, P03687, 6*e*−19)
orf23	12444	13901	485	DNA Helicase (*p*)	Replicative DNA helicase (phage P22, P03006, 2*e*−45)
orf24	13904	14398	163	DNA adenine methylase	Putative adenine-specific methylase (phage HP1, P51715, *e*−19)
orf25	14405	14902	165	Recombination protein (*ninB*)	Protein NinB (phage lambda, P03765, *e*−13)
orf26	14881	15126	81		
orf27	15126	15305	59		EF-Tu (*Photorhabdus luminescens* subsp. *lumondii* TTOI, NP_931892, 2*e*−07)
orf28	15280	15495	71		
orf29	15495	16064	189	Recombination endonuclease (*ninG*)	Protein NinG (phage lambda, P03770, 3*e*−18)
orf30	16064	16435	123	Antitermination protein (*q*)	Probable phage antitermination protein Q (*Yersina pestis* strain KIM, CAC90068.1, 7*e*−02)
orf31	16725	17621	298	Antirepressor (*ant*)	Antirepressor protein Ant (phage P22, P03037, 3*e*−26)
orf32	17785	18231	148		
orf33	18228	18596	122	Antiholin, holin 1?[b]	
orf34	18505	18870	121	Holin S, holin 2?[b]	
orf35	18939	19520	193	Lysin (*R*)	Putative prophage protein HI1415 (prophage ɸflu, H64028, 2*e*−56) Endolysin (bacteriophage epsilon15, NP_848233, 5*e*−29)
orf36	19523	19855	110	Rz lytic protein (*rz*)	Putative prophage protein HI1414 (prophage ɸflu, P44186, 7*e*−12) Putative Rz lytic protein (phage SfV, AAL89453.1, *e*−3)
orf37	19767	20048	93	Rz1 lytic protein (*rz1*)	Putative prophage protein HI1413 (prophage ɸflu, P44185, 3*e*−33)
orf38	20052	20318	88		
orf39	20475	21035	186	Terminase small subunit (*terS*)	Terminase small subunit (phage SF6, Q57374, 3*e*−11)
orf40	20980	22443	487	Terminase large subunit (*terL*)	Terminase large subunit (phage Beep781, AAN38019.1, *e*−50)
orf41	22445	23758	437		Putative prophage protein HI1409 (prophage ɸflu, P44183, 0.0)
orf42	23679	26081	800	Head protein	Putative prophage protein HI1407 (prophage ɸflu, B64122, *e*−109) Minor prohead-head protein (bacteriophage SPP1, NP_690662, 3.5*e*−02)
orf43	26078	26296	72		
orf44	26430	26630	66		
orf45	26713	27825	370		Putative prophage protein HI1405 (prophage ɸflu, AAC23055.1, *e*−171)
orf46	27839	28288	149		
orf47	28300	29214	304		Putative prophage proteins XF1577 and XF1682 (prophages XfP3 and XfP4, H82650, 7*e*−11)
orf48	29225	29584	119		Putative prophage proteins XF1579 and XF1684 (prophages XfP3 and XfP4, A82665, 2*e*−14)
orf49	29585	30031	148		Putative prophage proteins XF1580 and XF1685 (prophages XfP3 and XfP4, C82651, 7*e*−11)
orf50	30028	30399	123		Putative prophage proteins XF1581 and XF1686 (prophages XfP3 and XfP4, C82665, *e*−17)
orf51	30185	30826	213		Putative prophage proteins XF1582 and XF1687 (prophages XfP3 and XfP4, E82651, 9*e*−12)
orf52	30831	32339	502		Putative prophage proteins XF1583 and XF1688 (prophages XfP3 and XfP4, F82651, *e*−123)
orf53	32389	32820	143		Putative prophage proteins XF1584 and XF1689 (prophages XfP3 and XfP4, F82665, 9*e*−46)
orf54	32820	33239	139		Putative prophage proteins XF1585 and XF1690 (prophages XfP3 and XfP4, H82651, 5*e*−20)
orf54-54′	32820	33403	194		Putative prophage proteins XF1585 and XF1690 (prophages XfP3 and XfP4, H82651, 1*e*−19)
orf55	33390	35498	702	Tail-length tape measure protein	Tail-length tape measure protein (*Clostridium tetani* strain E88, AAO35681, 8*e*−09)
orf56	35502	36269	255		Putative prophage protein XF1591 (prophage XfP3, B82661, *e*−37)
orf57	36280	36594	104		Hypothetical protein XF1628 (*X. fastidiosa* strain 9a5c, C82648, 2*e*−20)

TABLE 1—*Continued*

Gene	Start position	Stop position	Size (aa)[a]	Potential function (gene name)	Significant matches (source, accession no., e value)
orf58	36881	37735	284		Putative prophage protein XF1593 (prophage XfP3, D82661, $4e-78$)
orf59	37732	38376	214	Baseplate protein	Putative baseplate protein (phage phi-CTX, BAA36243.1, $3e-06$)
orf60	38373	38732	119		Putative prophage protein XF1701 (prophage XfP4, F82648, $9e-26$)
orf61	38763	39905	380		Putative prophage protein XF1704 (prophage XfP4, A82649, $5e-96$)
orf62	39905	40486	193		Putative prophage protein HI1404 (prophage ϕflu, P44179, $5e-08$)
orf63	40497	41981	494	Tail fiber protein	Putative prophage protein HI1403 (prophage ϕflu, P44178, $e-07$) Putative tail fiber protein H (phage 186, AAC34164.1, $2e-04$)
orf64	41972	42592	206	Tail fiber assembly protein	Tail fiber assembly protein P37 (phage SfV, O22005.1, $2e-04$)
orf65	42573	42833	86		Hypothetical protein NMB1120 (*Neisseria meningitidis* strain MC58, B81120, $4e-24$)

[a] aa, amino acids.

[b] ?, assignment is speculative (see the text for a discussion).

two putative promoter sequences, which may correspond to the promoters for repressor maintenance (P_{RM}) and to $P_{R'}$, are evident in the *cI* and *cro* intergenic region of AaΦ23 (data not shown). Thus, AaΦ23 is thought to use a lysogeny control system similar to that of phage lambda (26).

The deduced gene product of *orf20* may be a antirepressor protein similar to the one named AntB in *E. coli* phage N15, thought to play a role in anti-immunity (28). *orf20* is also closely related to gene HI1422 of the prophage ϕflu (Table 1). *orf31* codes for a second putative antirepressor belonging to the protein family of Ant that is expressed in several phages and has been extensively characterized for P22 (31). The gene product of *orf30* shares significant homology with a probable phage antitermination protein Q (Table 1). Finally, no homologies to other regulatory proteins, such as cII, cIII, or antitermination protein N, have been identified on the AaΦ23 genome.

The DNA replication machinery of AaΦ23 (including ORFs 22 and 23, encoding phage P22 DNA replication protein and P22 DNA helicase homologues, respectively) seems to be closely related to that of the lambdoid bacteriophages (35).

ORFs 25 and 29 show homologies to the NinB and NinG proteins, respectively, of several phages (Table 1). In lambda, these two genes are known as *orf* (*ninB*) and *rap* (*ninG*), two recombination genes located in the *ninR* region. A recent study showed that they participate in Red recombination, the primary pathway operating when wild-type lambda grows lytically in Rec$^+$ cells (34). Additional experiments are needed to determine whether AaΦ23 induces a similar pathway when it grows lytically in *A. actinomycetemcomitans*.

In contrast to lambda, AaΦ23 codes for a DNA adenine methylase (ORF24) (Table 1). When *orf24* was introduced into the Dam$^-$ *E. coli* strain GM48, the genomic DNA was resistant to digestion by MboI but not by Sau3AI, indicating that it was methylated on the adenine residue of specific GATC sequences (data not shown). Also, a host-encoded *A. actinomycetemcomitans* DNA adenine methylase has been shown to be active in vivo (8). DNA methylases participate in regulatory events of DNA replication, methyl-directed mismatch repair, and transposition (20). These enzymes are also associated with bacterial DNA restriction-modification systems that are responsible for the degradation of foreign DNA such as conjugative plasmids, transposons, or bacteriophage DNA (29). It has been speculated that some bacteriophages express their

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>attL of A. actinomycetemcomitans strain ZIB1023
TAGCTCAGTTGGGAGAGCGCTTGA ATGGCATTCAAGAGGTCGTCGGTTCGATCCCGATTATCTCCACCAAATT TTATAGCTTTATCAATTAGTTAGTTATTGATA
                                                                                                      *  N  N  I
>attR of A. actinomycetemcomitans strain ZIB1023
TGATTTATGTATAGGCTTTCAAAT ATGGCATTCAAGAGGTCGTCGGTTCGATCCCGATTATCTCCACCAAATT CCAAAATCCTATCAATAAAAATTGATAGGATT

>attP of bacteriophage AaΦ23
TGATTTATGTATAGGCTTTCAAAT ATGGCATTCAAGAGGTCGTCGGTTCGATCCCGATTATCTCCACCAAATT TTATAGCTTTATCAATTAGTTAGTTATTGATA
                                                                                                      *  N  N  I
>attB of A. actinomycetemcomitans strain HK1651
TAGCTCAGTTGGGAGAGCGCTTGA ATGGCATTCAAGAGGTCGTCGGTTCGATCCCGATTATCTCCACCAAATT CCAAAATCCTATCAATAAAAATTGATAGGATT

>attL of A. actinomycetemcomitans strain ZIB1515
TAGCTCAGTTGGGAGAGCGCTTGA ATGGCATTCAAGAGGTCGTCGGTTCGATCCCGATTATCTCCACCAAATT TTATAGCTTTATCAATTAGTTAGTTATTGATA
                                                                                                      *  N  N  I
>attR of A. actinomycetemcomitans strain ZIB1515
TGATTTATGTATAGGCTTTCAAAT ATGGCATTCAAGAGGTCGTCGGTTCGATCCCGATTATCTCCACCAAATT CCAAAATCCTATCAATAAAAATTGATAGGATT

>attB of A. actinomycetemcomitans strain ZIB1015
TAGCTCAGTTGGGAGAGCGCTTGA ATGGCATTCAAGAGGTCGTCGGTTCGATCCCGATTATCTCCACCAAATT CCAAAATCCTATCAATAAAAATTGATAGGATT

>attB of A. actinomycetemcomitans strain ZIB1001
TAGCTCAGTTGGGAGAGCGCTTGA ATGGCATTCAAGAGGTCGTCGGTTCGATCCCGATTATCTCCACCAAATT CCAAAATCCTATCAATAAAAATTGATAGGATT

>attB of H. influenzae strain Rd KW20
TAGCTCAGTTGGGAGAGCGCTTGA ATGGCATTCAAGAGGTCGTCGGTTCGATCCCGATTATCTCCACCAAATT AATAAAGCAAGGTAAAGCACCTTGCTGTTTTA
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FIG. 2. *attP*, *attL*, *attR*, and *attB* sites on the bacteriophage AaΦ23 genome and on several bacterial genomes. The *att* core sequence is boxed. The C-terminal end of the putative integrase is underlined. The asterisk represents the stop codon of the integrase gene.

own DNA adenine methylase to overcome this bacterial protection. Some DNA adenine methylases also play a role in regulating bacterial virulence (12). Nevertheless, the involvement of these enzymes in the regulation of *A. actinomycetemcomitans* virulence genes remains to be studied.

AaΦ23 probably uses the same lysis strategy as described for many double-stranded DNA phages (41). The genetic organization (SRRzRz1) of the putative lysis cassette, coded by *orf34* to *orf37*, is closely related to the one generally described for lambdoid phages. None of the AaΦ23 ORFs share homologies with a holin (S), but ORF34 may be a good candidate because (i) it is topologically similar to the T4 holin (27; data not shown), (ii) it is localized upstream of *R*, and (iii) ORF34 may correspond to HI1416 of φflu (14). In fact, the RRzRz1 part of the AaΦ23 putative lysis cassette is closely related to the *H. influenzae* prophage Φflu (Table 1). Interestingly, when scanned for protein domains (http://hits.isb-sib.ch /cgi-bin/PFSCAN), HI1416 was found to match the phage holin 3 family ($4e-66$), which also includes the lambda holin S.

Nevertheless, no dual start motif (4) is present on the sequence of *orf34*, suggesting that an antiholin function, if present, may be coded by a separate gene. It would not be surprising that ORF33 plays a role in lysis, as it also represents a putative membrane protein sharing the same organization and charge distribution as ORF34 (data not shown). Additional experiments are required to characterize the AaΦ23 holin system. *orf35* probably codes for the lysin (R), which could represent a new lysozyme (glycosylase), as this protein shares strong homologies with several bacteriophage lytic enzymes, putative lysozymes, and chitinases (Table 1 and data not shown). Moreover, this ORF shares homologies with the glycoside hydrolase 19 protein family (PFAM accession number PF00182, $3.7e-2$), which groups proteins with chitinase activity. Some chitinases have been shown to display a lysozyme activity (22). Nevertheless, it is not clear whether ORF35 is a true lysozyme or whether it is capable of transglycosylation, as reported for the endolysin gp*R* of phage lambda (2). *orf36*, which is located directly downstream of *orf35*, presents weak homologies to the putative Rz lytic protein expressed by the *Shigella flexneri* bacteriophage SfV (Table 1). Moreover, *orf36* overlaps with *orf37*, the 93-amino-acid gene product of which harbors a prokaryotic lipoprotein motif (Prosite accession number PS00013) between residues 17 and 27 and may, therefore, represent the AaΦ23 homologue of the Rz1 prolipoprotein. In many phages of gram-negative hosts, Rz and Rz1 play a role as auxiliary lytic proteins that are thought to interact with the outer membrane or with its links to the peptidoglycans (41).

orf39 and *orf40* are possibly coding for the small and large subunits of the terminase enzyme, respectively (Table 1). This holoenzyme is required for packaging of the phage genomic DNA into the empty capsid shells (6). In AaΦ23, the phage DNA has been shown to be circularly permuted and terminally redundant for approximately 1.6 kb (37), and it may therefore be packaged by the headful mechanism. Thus, the AaΦ23 terminase complex may cut concatemeric DNA molecules, resulting from rolling circle replication, at a unique *pac* site to start the first round of packaging. Taking into consideration the 1.6-kb terminal redundancy, about 104% of the genomic DNA molecule is packaged into phage heads on subsequent rounds of packaging.

Few genes coding for structural components were identified on the AaΦ23 genome. The gene products of *orf42*, *orf55*, *orf63*, *orf64*, and *orf59* seem to be elements of the head, tail, tail fibers, and baseplate, respectively, of the AaΦ23 phage particle (Table 1). ORF42 contains a phage Mu protein F-like domain, from amino acid 156 to 262, found in members of a family representing possible minor phage head proteins (PFAM accession number PF04233, *e* value of $2.7e-30$). It shows significant amino acid sequence homologies to one member of this family, the gene 7 protein of bacteriophage SPP1, which has been previously described as being required for viral head morphogenesis (1). Programmed translational frameshifts occur in the tail gene operon of several bacteriophages, usually in the gene preceding the tape measure protein (7, 19). Indeed, a small additional orf (*orf54′*) is present upstream of *orf55* on the AaΦ23 genome (Fig. 1). This 99-amino-acid ORF starts with a cysteine in the -1 reading frame at 135 nucleotides before the end of *orf54* and extends 12 nucleotides into *orf55* (data not shown). Seventy-two nucleotides downstream of the first codon of *orf54′*, a stretch of seven T is found, which may be related to sites used for the -1 translational frameshift (10). Thus, *orf54′* may be translated as an extension of *orf54* following a -1 translational frameshift. The gene product of *orf54-orf54′* is predicted to be composed of 194 amino acids. Both, ORF54 and ORF54-ORF54′ have unknown functions.

BLAST analysis of the phage genomic sequence revealed two segments of strong homology over 8.7% of the AaΦ23 genome (average of 97% homology over 3,795 bp) with the *A. actinomycetemcomitans* strain HK1651 genomic sequence (ftp: //ftp.genome.ou.edu/pub/act) (Fig. 1). To determine whether these homologies are part of a degenerate prophage or some other modularly related prophage, 40-kb genomic sequences flanking the region of homology on either side were analyzed. Only four additional phage-related ORFs were identified among 80 potential ORFs detected. This indicates that this genomic region of *A. actinomycetemcomitans* strain HK1651 contains an AaΦ23-related prophage remnant rather than an intact prophage. Therefore, it was not surprising that we were not able to induce phage production from *A. actinomycetemcomitans* strain HK1651 by mitomycin C treatment.

A. actinomycetemcomitans is most closely related to *Haemophilus aphrophilus* and other members of the family *Pasteurellaceae* (3) while the plant pathogen *Xylella fastidiosa* is also a gammaproteobacterium (family *Xanthomonadaceae*) more distantly related to *H. influenzae* (32). Ten AaΦ23 ORFs share homologies with ORFs of φflu (14) and are present in a very similar gene order on both genomes. 36% of the AaΦ23 ORFs (24 of 66) share homology with hypothetical proteins from *X. fastidiosa* (Table 1 and data not shown). Interestingly, 14 of these ORFs share homology and show a similar gene order to ORFs contained in *X. fastidiosa* strain 9a5c prophages XfP3 and/or XfP4 (Table 1 and data not shown). These observations suggest that AaΦ23 is related to φflu and XfP3/XfP4 and that they could have had common ancestor(s).

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been assigned accession number AJ560763 in the EMBL database.

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