Title: Isolation and characterization of bacteriophages infecting *Staphylococcus epidermidis*

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Short title: *Staphylococcus epidermidis* phages

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Abstract

Bacteriophages infecting *Staphylococcus epidermidis* were isolated by mitomycin C induction. Three distinct phages (vB_SepiS-phiIPLA5, vB_SepiS-phiIPLA6 and vB_SepiS-phiIPLA7) -defined by plaque morphology, structure, virion proteins pattern, DNA restriction bands and host range- were obtained. One-step growth curves of bacteriophages under optimal growth conditions for *S. epidermidis* F12 revealed eclipse and latent periods of 5-10 min and 10-15 min respectively, with burst sizes of about 5 to 30 PFU per infected cell. Transmission electron microscopy revealed that the phages were of similar size and belonged to the *Siphoviridae* family. Phage phi-IPLA7 had the broadest host range infecting 21 out of 65 *S. epidermidis* isolates. Phage phi-IPLA5 seemed to be a virulent phage probably derived from phi-IPLA6. Phages phi-IPLA5 and phi-IPLA7 exhibited increasing plaques surrounded by a halo that could be indicative of a polysaccharide depolymerase activity. Viable counts, determined during the infection of *S. epidermidis* F12, confirmed that phi-IPLA5 had a potent lytic capability and reduced *S. epidermidis* population by 5.67 log-units in 8 h of incubation; in the presence of the mixture of phi-IPLA6 and phi-IPLA7, however, a reduction of 2.27 log units was detected

Introduction

Staphylococcus epidermidis was previously regarded as an innocuous commensal microorganism on the human skin. However, this bacterium is now seen as an important opportunistic pathogen involved in balancing epithelial microbiota and as a major cause of nosocomial infections. This microorganism predominantly colonizes the mucous membranes as well as the cutaneous system of human body, but it can also cause infections in

immunocompromised individuals, in patients with implanted medical devices or even in healthy women, where the staphylococci penetrate cutaneous and mucosal barriers (13, 19, 5). In the animal health context, *S. epidermidis* remains as one of the most commonly isolated bacteria responsible for bovine mastitis (16).

Biofilm formation is a key factor in the infection process and is considered the most important virulence factor of *S. epidermidis*. It allows the adhesion to host tissues and increases antibiotic tolerance (17). The widespread use of various antimicrobial agents, including penicillins, macrolides, aminoglycosides, and semisynthetic penicillins such as methicillin, has led to the emergence of multiple-drug-resistant *S. epidermidis* strains (9). Furthermore, the ubiquity of *S. epidermidis* as a human commensal microorganism renders this bacterium an optimal carrier and reservoir for antibiotic resistance genes (18). As a result, there is a renewed interest to discover other natural antimicrobial agents as an alternative or supplementary treatment for infectious diseases.

Bacteriophages have very effective bactericidal activity and several advantages over other antimicrobial agents. Most notably, phages replicate at the expense of infectious bacteria, are available in abundance where they are most required, and so far, no serious or irreversible side effects of phage therapy have been described (21). Although there are no phage therapy products in the Western countries market at the moment numerous companies have developed or are in the process of developing phage-based products against *Pseudomonas* and *Staphylococcus aureus* infections (23, 14). Bacteriophages have also been used to reduce the catheter- associated biofilms of *S. epidermidis* strains (3).

To date, bacteriophages infecting *S. epidermidis* had been exclusively used for typing *S. epidermidis* strains (22). As such, the complete genome and molecular characterization of only two bacteriophages have been reported (4). Based on the scarce knowledge on *S. epidermidis* bacteriophages and the future prospects of phage therapy against this microorganism, the purpose of the present study was to isolate and characterize novel phages

able to infect *S. epidermidis* and to determine their lytic ability under lab-controlled conditions.

Material and Methods

Bacterial strains and growth conditions

Sixty-five *S. epidermidis* strains were isolated from women's breast milk (5), with 41 of them suffering infectious mastitis (Table 1). Staphylococcal cells were isolated in Agar Baird Parker (BP) and routinely cultured in TSB broth (Triptona Soy Broth, Scharlau) at 37°C with shaking or in TSB plates containing 2% (w/v) bacteriological agar (TSA).

Bacteriophage isolation

Strains were grown to exponential phase and subsequently induced by adding mitomycin C (0.5 μg/ml). After incubation at 37°C for 3 h with shaking, induced cultures were centrifuged at 16,100 × g for 5 min and the supernatants were filtered. The supernatants (5 μl) were spotted into agar overlay lawns of all the staphylococcal strains and monitored for zones of clearing. Plaques were re-isolated, propagated, and stored at −80°C in SM buffer (20 mg l^{-1} Tris HCl, 10 mg l^{-1} $MgSO_4$, 10 mg l^{-1} $CaCl_2$, 100 mg l^{-1} NaCl, pH 7.5) containing 50% glycerol (vol/vol). Concentrated and purified phage preparations were obtained from 1 liter of *S. epidermidis* F12 which was infected with the different phages at a multiplicity of infection (MOI) of 1. The infected cultures were then incubated for 3 h at 37ºC with vigorous shaking. Phages were further purified by a CsCl continuous density gradient (20).

Bacteriophage host range

The host range of phages was determined by the spot test. 5 μl of concentrated phage lysate (>10^9 PFU ml^{-1}) was dropped onto a TSB plate overlaid with *S. epidermidis* (10^8 CFU ml^{-1}). The host range was confirmed by the plaque assay. A 0.1 ml volume of stationary-phase host

culture (10^8 CFU ml^{-1}) was mixed with several dilutions of individual phage suspensions in 3 ml of molten TSB top agar (0.7% agar) and the mixture was poured on TSA plates. Efficiency of plaque formation (EOP) of selected phages was determined by dividing the phage titre on the test strain by the phage titre on the reference strain *S. epidermidis* F12. This strain was selected because it is infected by all the isolated phages.

Single-step growth curve

A standardized protocol (10) was adapted for the *S. epidermidis* phages. Curves were performed in TSB broth supplemented with $Ca(NO_3)_2$ (10 mmol l^{-1}) and $MgSO_4$ (10 mmol l^{-1}) using a MOI of 0.1. A mid-exponential-phase culture (10 ml) of *S. epidermidis* F12 (OD_{600nm} 0.1) was harvested by centrifugation and suspended into 0.1 volume of fresh TSB (ca. 10^7 CFU ml^{-1}). The phage was added and allowed to adsorb for 10 min at 37°C. The mixture was then centrifuged, pelleted cells were resuspended into 10 ml of TSB, and incubation continued at 37°C. Two set of samples were first taken at 5-min intervals for a period of 30 min, and subsequently at 15-min intervals. The first set of samples was immediately diluted and plated for phage titration. To determine the eclipse period, a second set of samples was treated with 1% (vol/vol) chloroform to release intracellular phages before phage titration.

Temperate *versus* lytic phage determination

To determine whether a phage was temperate or not, putative lysogens (resistant to infection) were isolated as previously described (7). Briefly, isolated colonies were recovered from lysis plaques and challenged with the corresponding phage to confirm resistance. Additionally, induction with mitomycin C further corroborated the presence of the prophage.

Electron microscope examination

Phage particles were negatively stained with 2% uranyl acetate, and electron micrographs were taken using a JEOL 12.000 EXII transmission electron microscope (JEDL USA Inc, Peabody, MA, USA).

Bacteriophage DNA isolation and restriction

Phage DNA was extracted by treatment of pure stocks as previously described (20). DNA was digested with restriction enzymes according to the supplier instructions (Takara Bio Inc., Japan).

Proteomic analysis of virion proteins

Phage structural proteins were extracted, purified as described (6) and analyzed by SDS-PAGE as described by Laemli (12) in a Miniprotean III (Bio-Rad, Richmond, CA) at a constant current of 30 mA. After electrophoresis, the gels were either stained with Coomassie R-250 blue or silver (Silver staining kit, protein, GE Healthcare Piscataway, NJ, USA).

Bacteria-phage challenge test against *S. epidermidis*

The bactericidal effect of phages on *S. epidermidis* F12 was observed by determining bacteria viable counts throughout the incubation period. 10 ml of TSB broth were inoculated with 1% (vol/vol) overnight *S. epidermidis* F12 culture and incubated at 37ºC with shaking until it reached early logarithmic phase (OD_{600}=0.1) (10^7 CFU ml^{-1}). A 100-fold dilution of the culture (10^5 CFU ml^{-1}) was infected and incubated to 37ºC. Phages were added at indicated MOIs and viable cells and phage titre were monitored at 2 h intervals for 8 h.

Results and Discussion

Isolation of *S. epidermidis* bacteriophages

Based on the renewed interest in phage therapy and the success of a number of recent animal experiments conducted with viable phage particles as antibacterial agents, we aimed to isolate

phages which could be tentatively useful as novel strategies to combat *S. epidermidis* infections. As potential hosts, several *S. epidermidis* strains of human origin (5) were selected (Table 1). Out of 65 genetically-diverse strains, 41 were isolated from the breast milk of women suffering from mastitis infection and 24 were isolated from healthy women. These two *S. epidermidis* populations clustered mainly into two distinct PFGE profiles, matching with the origin of the strains, and with pathogenic strains showing higher biofilm production and resistance to antibiotics (5). All the attempts to isolate bacteriophages infecting *S. epidermidis* strains from environmental samples such as breast milk of healthy and mastitic women, skin and mucous surface exudates were unsuccessful (data not shown). Consequently, mitomycin C induction of *S. epidermidis* strains was performed and the presence of bacteriophages in the culture supernatants tested. Two phages -phi-IPLA6 and phi-IPLA7- were isolated from *S. epidermidis* DD2Laa and *S. epidermidis* AEA1 strains, respectively. Furthermore, when the bacteriophage phi-IPLA6 was propagated on a lawn of *S. epidermidis* F12, some clear lysis plaques were observed. The plaques were further purified and the putative lytic phage was named phi-IPLA5. Based on these results, the yield of mitomycin C inducible prophages in *S. epidermidis* is rather low (3%). However, the apparently low presence of prophages could be due to the lack of appropriate sensitive host strains to detect them. Therefore, it would be premature to anticipate a low prophage content in this particular species.

Host range of the isolated bacteriophages

The ability of new isolated phages to lyse pathogenic and commensal *S. epidermidis* strains was assayed by the spot test (Table 1). Phage suspensions produced an inhibition halo on 25 out of the 65 strains tested. However, plaques were not always observed when the phage suspensions were serially diluted. For example, phage phi-IPLA5 inhibited 17 strains but clear plaques were only observed on 5 of them. The same thing occurred with the other two

phages. This result could be explained by the effect of “lysis from without” caused by phages at high multiplicities of infection (MOI). In this case, cell lysis is produced by peptidoglycan hydrolases present in the mature virions of some phages (15). These muralytic activities locally degrade the peptidoglycan of the host cell in order to facilitate the entry of phage DNA during infection. Besides the distinct host range, the individual *S. epidermidis* phages showed differences up to 6 orders of magnitude in their infection effectiveness against sensitive *S. epidermidis* strains as judged by EOP. Furthermore, plaques formed by phages phi-IPLA6 and phi-IPLA7 were turbid while clear plaques in all sensitive strains were obtained with phi-IPLA5 (data not shown). Among the strains, only three (*S. epidermidis* F12, LCO17, and LV5RB3) were sensitive to the three bacteriophages and lysis plaques were observed (Table 1). Resistance to these phages might be due to superinfecction immunity by resident prophages not detected during the screening with mitomycin C. In addition, bacteriophages usually show a narrow host range and actually they are commonly used for identification of closely related bacterial strains (22). Because of this, further experiments are needed to isolate new phages and combine them to create an optimal cocktail with broad activity against *S. epidermidis* strains.

Absence of cross-immunity among bacteriophages phi-IPLA5, phi-IPLA6 and phi-IPLA7

The lytic nature of phi-IPLA5 was experimentally confirmed because lysogenic bacteria could not be isolated (data not shown). On the contrary, lysogenic cultures carrying phi-IPLA6 and phi-IPLA7 phages could be generated. Lysogenized strains become immune to infection by similar phages and might hinder the use of phages as therapeutic agents. In order to minimize this trouble, the use of several phages belonging to a distinct immunity group is convenient. *S. epidermidis* F12-phi-IPLA6 was resistant to infection by phage phi-IPLA6 but was infected and lysed by phages phi-IPLA5 and phi-IPLA7. Likewise, *S. epidermidis* F12-phi-IPLA7 cells were immune to phi-IPLA7 but susceptible to phi-IPLA5 and phi-IPLA6 infection (data

not shown). Based on these results, the temperate phages phi-IPLA6 and phi-IPLA7 belong to a distinct immunity group and, thus, suitable mixtures of these phages would prevent the development of lysogenic derivative strains.

Increasing size on lysis plaques surrounded by a halo

An interesting result was that phages phi-IPLA5 and phi-IPLA7 showed lysis plaques surrounded by halos that increased along longer incubations. These lysis plaques increased in size when they were kept on the lab-bench for 240 h (Fig. 1). Phage phi-IPLA5 plaques increased by 1.5 mm in 120 h while phi-IPLA7 plaques were 3 mm larger after 168 h. The plaque expansion and the presence of halos could be indicative of soluble enzymes degrading extracellular polymeric structures such as exopolysaccharides from the host strain. Previous studies have showed that certain *Klebsiella pneumoniae* and *Enterobacter agglomerans* phages synthesised an enzyme that was released from the infected bacteria during plaque formation (11). Phages producing depolymerases have biotechnological applications as treatments to prevent or control infectious biofilms (3). Therefore, considering that *S. epidermidis* is able to produce biofilms, identification of the putative depolymerase activity in phages phi-IPLA5 and phi-IPLA7 deserves further investigation.

Single-step growth curve

The proliferation rate of bacteriophages is usually determined by the latent period and the burst size. Both parameters can be calculated from the one-step growth curves. Data obtained from bacteriophages phi-IPLA5, phi-IPLA6 and phi-IPLA7 propagated on *S. epidermidis* F12 showed similar eclipse and latent periods (Fig. 2). The burst sizes ranged from 5 to 30 PFU per infected cell under the assay conditions. No data are available in other *S. epidermidis* phages; they were, however, similar to that for *Staphylococcus aureus* phages (7).

Morphology of phage particles

Concentrated and purified solutions of phages phi-IPLA5, phi-IPLA6 and phi-IPLA7 were examined by electron microscopy (Fig. 2). A number of shared features were observed: an isometric capsid, a long and narrow non-contractil tail, as well as the presence of both a baseplate and short tail fibers. Based on its morphology, these bacteriophages belong to the family *Siphoviridae*. The diameters of the capsids (means ± standard deviations) of phi-IPLA5, phi-IPLA6 and phi-IPLA7 were, respectively, 53.04 ± 4.95, 47.42 ± 6.27, and 53.08±10.66 nm. The tail of phi-IPLA5 had a length of 144.51± 10.07 nm and width of 13.07± 2.08 nm, and was the largest compared to phi-IPLA6 (135.87 ± 9.58 nm long and 12.54± 1.66 nm wide) and phi-IPLA7 (136.68 ± 8.68 nm long and 9.81± 0.87 nm wide) tails.

SDS-PAGE and genetic analysis of bacteriophages

In phage phi-IPLA5 four structural proteins were observed, and the most abundant polypeptide had a molecular mass about 34 kDa. Two polypeptides with molecular masses of about 30 and 34 kDa were observed in phi-IPLA6. Finally, two main bands (27.5 and 34 kDa) and at least 3 other polypeptide bands ranging from ca. 21 to 76 kDa were also detected in phi-IPLA7 (Fig. 3A). These bands, which were easily detected, were most likely the major head and tail proteins. In order to examine whether phages were genetically different, restriction analyses of their genomic DNA were performed. The estimated sizes of the full-length phage genomes of phi-IPLA5, phi-IPLA6 and phi-IPLA7 were, respectively, 39 kb, 38 kb, and 33 kb. The restriction patterns of the phi-IPLA7 genome were unique (Fig. 3D). By contrast, phages phi-IPLA5 and phi-IPLA6 appeared to be very closely related as the restriction profiles were very similar. The only significant difference resided in the absence of *Pst*I fragments in phi-IPLA6 (Fig. 3B and 3C). However, as has been showed above, phage phi-IPLA5 was able to infect the lysogenic strain *S. epidermidis* F12-phi-IPLA6. Thus, it could be speculated that phi-IPLA5 is a virulent derivative from phi-IPLA6, i.e. a phage able

to overcome superinfection immunity. Point mutations in the operator regions could inhibit the specific binding of the resident prophage repressor which allows the replication of the infecting phage (2).

Bacteriophage inhibition of *S. epidermidis* F12 growth

Preliminary challenge trials were performed to evaluate the potential of the isolated phages as antimicrobials against *S. epidermidis.* The mixture of the temperate phages phi-IPLA6 and phi-IPLA7 was used at MOI = 10 to infect *S. epidermidis* F12 (Fig. 4A). Within the first 4 h, viable counts were similar to phage-infected and uninfected (control) cultures, and staphylococcal proliferation was prevented afterwards. *S. epidermidis* counts were reduced by 2.27 log units compared with the control cultures (Fig. 4A). Furthermore, viable bacteria were still at 10^6 CFU ml^{-1} at 24 h (data not shown). It was expected that the addition of a mixture of the two temperate phages to *S. epidermidis* cultures would suppress bacterial growth and even fully lyse the host culture since they do not belong to the same immunity group. However, even though phage multiplication took place, the phage mixture failed to completely eliminate the host cells. It is possible that the proper phage:bacteria host ratio was not reached under these experimental conditions (1). *S. epidermidis* F12 was also infected with the lytic phage phi-IPLA5 at MOI = 150. A similar trend of viable bacteria was observed within the first 2 h. The infected cultured stopped growing for the next 4 h and then viable counts dropped drastically. At the end of the incubation period, a difference of 5.67 log units was observed between phage-infected and control cultures (Fig. 4B). This result supports the “general” assumption that lytic phages would be more suitable for phage therapy. Nevertheless, in a phage therapy context, further characterization of these newly isolated *S. epidermidis* phages, will be carried out as a pre-requisite to examine their safety, i.e. absence of virulent traits. Moreover, genome mining might reveal novel lytic proteins and, hopefully, other relevant antimicrobials active in biofilms such as depolymerases.

References

1. Cairns B, Timms AR, Jansen VA, Connerton IF, Payne RJ (2009) Quantitative models of *in vitro* bacteriophage-host dynamics and their application to phage therapy. PLoS Pathog 5:e1000253

2. Carlson NG, Little JW (1993) A novel antivirulence element in the temperate bacteriophage HK022. J Bacteriol 175:7541-7549

3. Curtin JJ, Donlan RM (2006) Using bacteriophages to reduce formation of catheter-associated biofilms by *Staphylococcus epidermidis.* Antimicrob Agents Chemother 50: 1268-1275

4. Daniel A, Bonnen PE, Fischetti VA (2007) First complete genome sequence of two *Staphylococcus epidermidis* bacteriophages. J Bacteriol 189:2086-2100

5. Delgado S, Arroyo R, Jimenez E, Marin ML, Del Campo R, Fernandez L, Rodriguez JM (2009) *Staphylococcus epidermidis* strains isolated from breast milk of women suffering infectious mastitis: potential virulence traits and resistance to antibiotics. BMC Microbiol 9:82

6. García P, Ladero V, Suárez JE (2003) Analysis of the morphogenetic cluster and genome of the temperate *Lactobacillus casei* bacteriophage A2. Arch Virol 148:1-20

7. García P, Madera C, Martínez B, Rodríguez A, Suárez JE (2009) Prevalence of bacteriophages infecting *Staphylococcus aureus* in dairy samples and their potential as biocontrol agents. J Dairy Sci 92:3019-3026

8. García P, Martínez B, Obeso JM, Lavigne R, Lurz R, Rodríguez A (2009) Functional genomic analysis of two *Staphylococcus aureus* phages isolated from the dairy environment. Appl Environm Microbiol 75:7663-7373

9. Gill SR, Fouts DE, Archer GL, Mongodin EF, Deboy RT, Ravel J, Paulsen IT, Kolonay JF, Brinkac L, Beanan M, Dodson RJ, Daugherty SC, Madupu R, Angiuoli SV, Durkin AS, Haft

DH, Vamathevan J, Khouri H, Utterback T, Lee C, Dimitrov G, Jiang L, Qin H, Weidman J, Tran K, Kang K, Hance IR, Nelson KE, Fraser CM (2005) Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. J Bacteriol 187:2426-2438

10. Herrero M, de los Reyes-Gavilán CG, Caso JL, Suárez JE (1994) Characterization of ϕ393-A2, a bacteriophage that infects *Lactobacillus casei*. Microbiology 140:2585-2590

11. Hughes KA, Sutherland IW, Clark J, Jones MV (1998) Bacteriophage and associated polysaccharide depolymerases - novel tools for study of bacterial biofilms. J Appl Microbiol 85:583-590

12. Laemmli U (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685

13. McCann MT, Gilmore BF, Gorman SP (2008) *Staphylococcus epidermidis* device-related infections: pathogenesis and clinical management. J Pharm Pharmacol 60:1551-1571

14. Merabishvili M, Pirnay JP, Verbeken G, Chanishvili N, Tediashvili M, Lashkhi N, Glonti T, Krylov V, Mast J, Van Parys L, Lavigne R, Volckaert G, Mattheus W, Verween G, De Corte P, Rose T, Jennes S, Zizi M, De Vos D, Vaneechoutte M (2009) Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS One 4:e4944

15. Moak M, Molineux IJ (2004) Peptidoglycan hydrolytic activities associated with bacteriophage virions. Mol Microbiol 51:1169-1183

16. Oliveira M, Nunes SF, Carneiro C, Bexiga R, Bernardo F, Vilela CL (2007) Time course of biofilm formation by *Staphylococcus aureus* and *Staphylococcus epidermidis* mastitis isolates. Vet Microbiol 124:187-191

17. Otto M (2008) Staphylococcal biofilms. Curr Top Microbiol Immunol 322:207-228

18. Otto M (2009) *Staphylococcus epidermidis*- the “accidental” pathogen. Nature Reviews 7:555-567

19. Piette A, Verschraegen G (2009) Role of coagulase-negative staphylococci in human disease. Vet Microbiol 134:45-54

20. Sambrook J, Maniatis T, Fritsch EF (1989) Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

21. Sulakvelidze A, Kutter E (2005) Bacteriophage therapy in humans. In *Bacteriophages: Biology and Application.* Kutter E, Sulakvelidze A., editors. pp. 381-436. Boca Raton, CRC Press

22. Talbot HWJr, Parisi JT (1976) Phage typing of *Staphylococcus epidermidis*. J Clin Microbiol 3:519-523

23. Wright A, Hawkins CH, Anggård EE, Harper DR (2009) A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. Clin Otolaryngol 34:349-357

Tables

Table 1. *Staphylococcus epidermidis*_bacteriophages selected along this study with their respective host range. The efficiency of plaquing (EOP) values are the mean of three different experiments (mean±standard deviation). *S. epidermidis* F12 was taken as the reference strain. *, inhibition halo.

1

2

S. epidermidis strain	Source	Bacteriophage			*S. epidermidis* strain	Source	Bacteriophage		
		phi-IPLA5	phi-IPLA6	phi-IPLA7			phi-IPLA5	phi-IPLA6	phi-IPLA7
CJ11	M	-	-	-	Z2LDC11	M	-	-	-
M121	M	-	-	-	Z2LDC12	M	-	-	-
V1LD1	M	-	-	-	Z2LDC14	M	-	-	-
CJ9	M	-	-	-	DG2ñ	M	-	-	-
PLD22	M	-	-	-	AQLD3	M	-	-	-
CJBP1	M	-	-	*	ASLI3	M	-	-	-
AEA1	M	*	-	-	ASLD1	M	-	-	*
ARLI1	M	-	-	-	ASLD3	M	-	-	$6.33x10^{-6}$± $6.23x10^{-7}$
K	M	-	-	*	LP222	H	-	-	*
ASLD2	M	-	-	-	LX5081	H	-	-	-
D623	M	-	-	-	LCC5092	H	-	-	-
DC2LAe	M	-	-	-	LCC5081	H	-	-	-
F12	M	1±0.02	1±0.15	1±0.09	LP223	H	-	-	-
CJBP3	M	-	-	-	LV221	H	-	-	-
AQLI2	M	-	-	-	LV222	H	-	-	-
B	M	*	-	-	LV521	H	-	-	-
B1CD2	M	-	-	-	LI5081	H	-	-	-
DD2Laa	M	*	-	-	LO5081	H	*	1.02±0.02	$1.15x10^{-6}$± $2.54x10^{-7}$
DF2Lbk	M	-	-	-	LO5082	H	*	-	-
DH3LIK	M	-	-	-	LV5081	H	-	-	-
C213	M	-	-	-	LG5082a	H	*	-	*
Z2LDC17	M	-	-	-	LG006	H	-	-	-
S1LDC13	M	-	*	*	LCO16	H	*	4.03±0.24	1.93±0.21
4GLI4	M	-	-	-	LCO17	H	$6.41x10^{-7}$± $0.82x10^{-8}$	0.47±0.03	1.07±0.16
YLIC16	M	*	-	*	LEO10	H	-	-	-
CJBP2	M	*	$4.28x10^{-4}$± $1.74x10^{-5}$	$6.31x10^{-6}$± $4.81x10^{-7}$	LEO11	H	*	-	*
CJBP3	M	-	-	-	LEO35	H	-	-	-
P2LD1	M	-	-	-	LG005	H	$5.06x10^{-6}$± $1.01x10^{-6}$	*	$1.04x10^{-6}$± $6.24x10^{-8}$
SILDC13	M	*	-	*	LX5RB3	H	-	-	*
YLIC13	M	0.46± 0.04	-	0.67±0.02	LX5RB4	H	-	-	-
YLIC14	M	-	-	-	LO5RB1	H	-	-	*
YLIC17	M	*	-	*	LV5RB3	H	$3.74x10^{-7}$± $3.97x10^{-9}$	0.32± 0.01	0.99±0.01
S1LDC18	M	-	-	-					

3 M, mastitic women

4 H, healthy women

Figures

Figure 1. Evolution of the plaque size throughout time on a lawn of *S. epidermidis* F12 at room temperature. (●) phage phi-IPLA5, (■) phage phi-IPLA6 and (▲) phage phi-IPLA7. Diameter values were expressed as the average of 85 plaques measurements.

Figure 2. Electron micrographs, one-step growth curves and growth parameters of phages A) phi-IPLA5, B) phi-IPLA6 and C) phi-IPLA7. In micrographs, scale bars are 100 nm. In graphs, symbols are the PFU per infected cell in chloroform-treated cultures (○) and the PFU/infected cell in untreated cultures (●). Each data is the mean of three experiments.

Figure 3. Structural proteins and restriction profile of genomic DNA of *S. epidermidis* phages. A) Analysis by SDS-PAGE electrophoresis and Coomassie staining of the structural proteins of 1) phi-IPLA5, 2) phi-IPLA6 and 3) phi-IPLA7 particles. Protein molecular size markers (kDa) are shown on the left (Lane L). DNA Restriction analysis of (B) phi-IPLA5, C) phi-IPLA6 and D) phi-IPLA7. Lanes 1: *Bam*HI. Lanes 2: *Eco*RI. Lanes 3: *Hind*III. Lanes 4: *Pst*I. L: 500 bp Molecular Ruler (Bio-Rad).

Figure 4. Growth of *S. epidermidis* F12 at 37°C in the presence of A) phi-IPLA6 and phi-IPLA7 (1:1) mixture at a MOI= 10 (■) and B) phi-IPLA5 at MOI= 150 (■). In both, A and B, growth of *S. epidermidis* F12 without phages is represented by CFU ml^{-1} (◆), and the total number of phages corresponds to PFU ml^{-1} (○). Each data point is a mean of three independent experiments.

Acknowledgments

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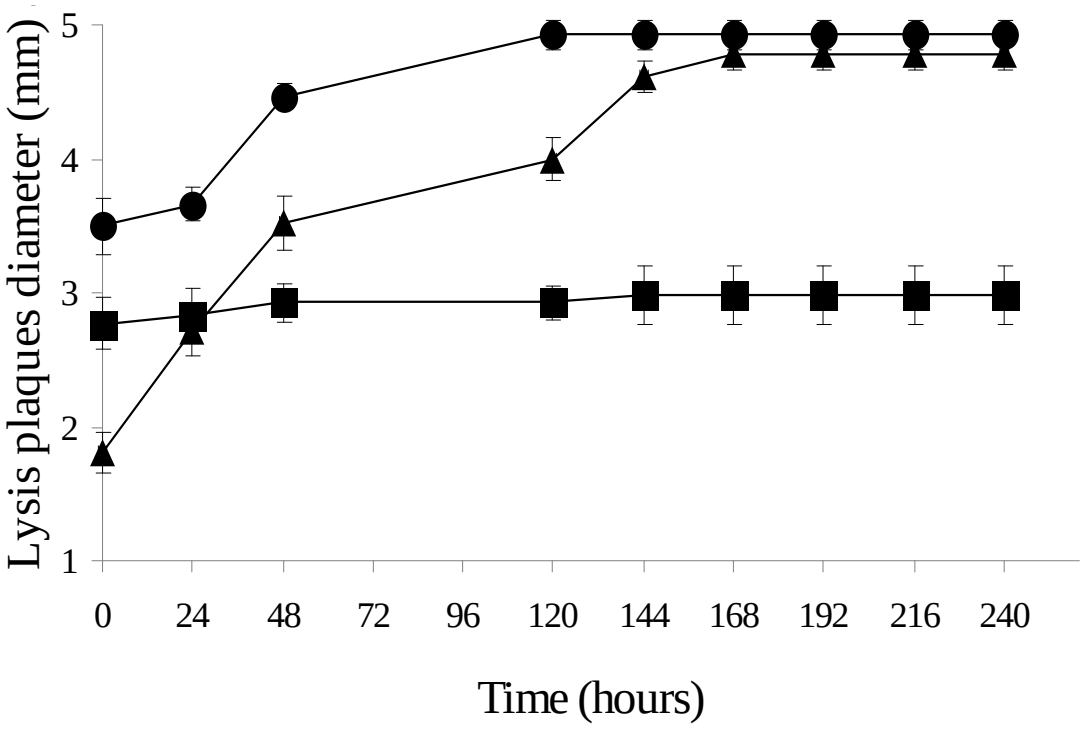
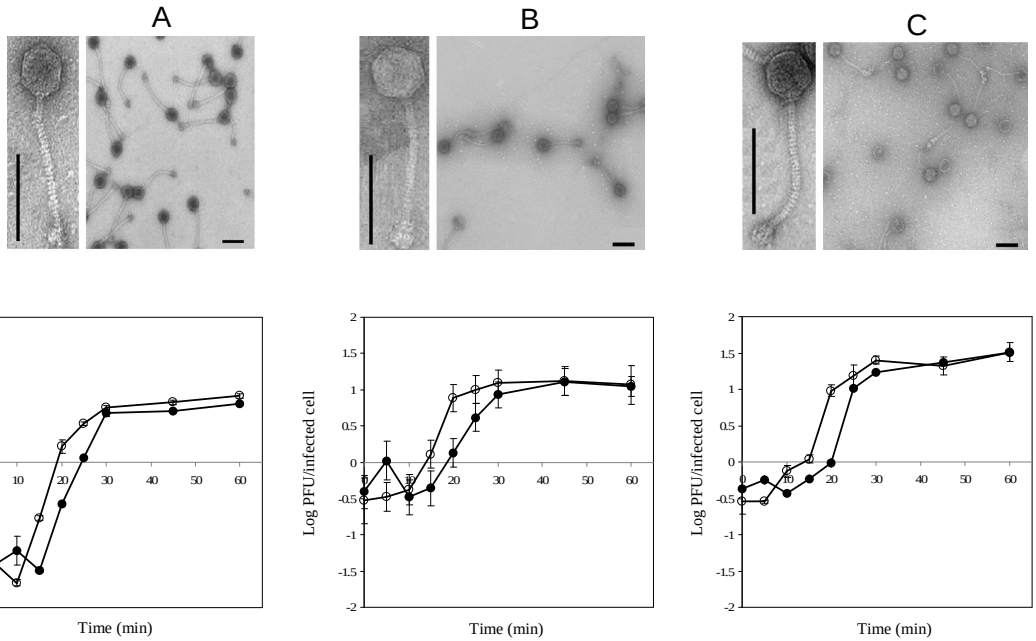
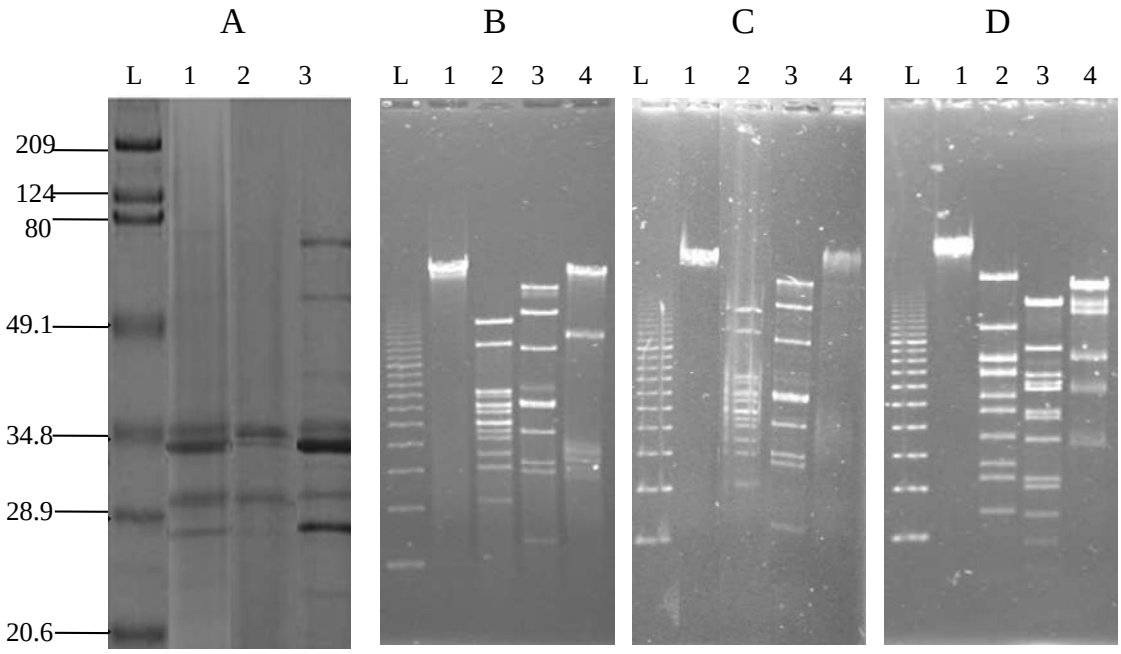

Lysis plaques diameter (mm)
1
2
3
4
5
0
24
48
72
96
120
144
168
192
216
240
Time (hours)

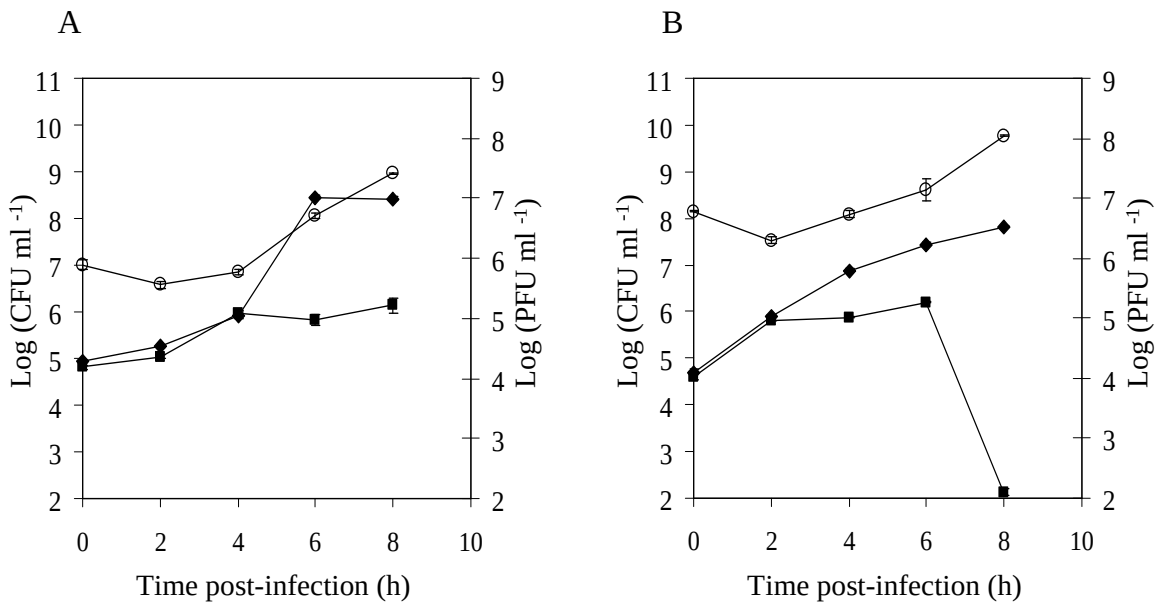


Parameter	phi-IPLA5	phi-IPLA6	phi-IPLA7
Eclipse period (min)	10	10	5
Latent period (min)	15	15	10
Burst size (phage/cell)	5-8	11-13	25-30


A
B
C
D
L 1 2 3
L 1 2 3 4
L 1 2 3 4
L 1 2 3 4
209
124
80
49.1
34.8
28.9
20.6

1

2


A
B
Log (CFU ml -1)
Log (PFU ml -1)
Time post-infection (h)
0
2
4
6
8
10
2
3
4
5
6
7
8
9
10
11