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Research Article

RESEARCH ARTICLE

Assessment of *Sep1virus* interaction with stationary cultures by transcriptional and flow cytometry studies

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One sentence summary: Description of a phage with the ability to initiate its lytic cycle immediately after infecting *S. epidermidis* stationary cultures assessed by flow cytometry and qPCR.

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ABSTRACT

The majority of phage infection studies are performed in bacteria that are growing exponentially, although in nature, phages usually interact also with non-replicating cells. These stationary-phase cells differ from exponential cells morphologically, physiologically and metabolically. The interaction of a *Sep1virus* with *Staphylococcus epidermidis* stationary and exponential phase cells was explored. Phage SEP1 efficiently infected both cell culture states, without the addition of any fresh nutrients to stationary cultures. Phage–host interactions, analysed by flow cytometry, showed stationary-phase cells response to phage immediately after SEP1 addition. Quantitative PCR experiments corroborate that phage genes are expressed within 5 min of contact with stationary phase cells. The increase of host RNA polymerase transcripts in stationary cells suggests that SEP1 infection leads to the upregulation of host machinery fundamental for completion of its lytic life cycle. SEP1 infection and replication process highlights its potential clinical interest targeting stationary phase cells.

Keywords: *Staphylococcus epidermidis*; bacteriophage; flow cytometry; stationary cells; gene expression; phage-host interactions

INTRODUCTION

Staphylococcus epidermidis is a usual commensal colonizer of the human skin and mucous membranes. This ubiquitous organism is currently regarded as an important nosocomial pathogen, since after the occurrence of an injury it can penetrate through the epithelium and becomes a threat, namely in immunocompromised patients (Cheung and Otto 2010). Moreover, this pathogen usually encodes specific antibiotic-resistance genes, namely against vancomycin and methicillin, which are used in first-line therapies against staphylococcal infections (Srinivasan, Dick and Perl 2002; Haque *et al.* 2011). Nowadays, the development of bacterial resistance to antibiotics is a major concern worldwide. Recently, WHO considered the emergence of multi-drug resistant pathogenic bacteria a major public health concern (WHO 2014) recommending researchers and pharmarceutical industries to focus on the development of new antimicrobials to combat antibiotic resistant pathogens (WHO 2017).

The use of bacteriophages (phages) to combat bacterial infections has been widely assessed, since several phages can target antibiotic-resistant bacteria (Gorski *et al.* 2016). Phages are viruses that are very specific towards their bacterial hosts,

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and consequently harmless to human cells and human natural microbiota (Sulakvelidze, Alavidze and Morris 2001). Studies of phage-host interactions are commonly conducted in models where bacterial hosts are growing on the exponential phase. However, these conditions are usually distinctive from those encountered in natural environments (Jones *et al.* 2007). Bacterial cells can persist for long periods under starved conditions without dividing (Kaprelyants, Gottschal and Kell 1993), being usually very tolerant to the majority of antimicrobials (Cerca *et al.* 2011a; Cerca *et al.* 2014). In laboratory conditions, active dividing cells and starved non-dividing cultures can be obtained in exponential and stationary cultures, respectively. Stationary phase cells are different from exponential phase cells both morphologically (Zhou and Cegelski 2012) and metabolically (Vandecasteele *et al.* 2001). Consequently, for the success of phage therapy, it is of utmost importance to understand how the differences found in bacterial cells physiology can affect phage infection.

In this study, we describe the activity of the previously isolated *S. epidermidis*-specific phage phiIBB-SEP1 (SEP1) (Melo *et al.* 2014) against *S. epidermidis* stationary phase cells. Phage-host interactions were analyzed through gene expression studies and a novel flow cytometry approach.

MATERIALS AND METHODS

Bacterial strains and culture conditions

The biofilm-forming strain *S. epidermidis* 9142 was used in this study (Mack, Siemssen and Laufs 1992). Bacteria were grown in Tryptic Soy Broth (TSB, VWR Chemicals, PA, USA), Tryptic Soy Agar (TSA; VWR Chemicals) or in TSA soft overlays (TSB with 0.4% agar) at 37°C. *Staphylococcus epidermidis*-specific phage SEP1 was previously isolated and characterized (Melo *et al.* 2014).

Phage production

SEP1 phage particles were produced as described before (Melo *et al.* 2014). Briefly, 100 μL of a phage suspension at 10^6 PFU mL^{-1} was spread on a *S. epidermidis* 9142 lawn, using a paper strip. After 16 h of incubation at 37°C, full lysis was checked. Then, 3–4 mL of SM Buffer [100 mM NaCl, 8 mM $MgSO_4$, 50 mM Tris/HCl (pH 7.5), 0.002% (w/v) gelatin] were added to each plate. Subsequently, plates were agitated at 120 rpm in an orbital shaker (BIOSAN PSU-10i, Riga, Latvia) for 24 h at 4°C. Thereafter, liquid and top agar were collected and centrifuged for 10 min, 10 000 *g*, 4°C and the supernatant was recovered.

Phage concentration was performed essentially as described by Sambrook and Russel (2001). The phage lysate was first concentrated with NaCl (5.84% (w/v)) and PEG 8000 (10% (w/v)), and then purified with chloroform (1:4 (v/v)) and further filtered through a 0.22 μm cellulose acetate membrane (GE Healthcare, Little Chalfont, UK). Samples were stored at 4°C until further use.

Infection of planktonic cells

The infection of planktonic cells in both exponential and stationary phases was performed essentially as described before (Cerca, Oliveira and Azeredo 2007). Briefly, in order to induce infection of stationary phase cells, *S. epidermidis* was grown for 48 h at 37°C and 120 rpm. Cell suspensions were diluted with the supernatant of the remaining culture (centrifuged media used to grow the cells) to obtain an optical density at 600 nm (OD_{600nm}) of approximately 0.6 (~6×10^8 CFU mL^{-1}). To infect cells in the exponential phase, 250 μL of the overnight grown culture were used to inoculate 25 mL of fresh TSB and bacteria allowed to grow until an OD_{600nm} of approximately 0.6. In all experiments, 100 μL of phage suspension (10^8 PFU mL^{-1}) were added to 100 μL of bacterial suspension (10^8 CFU mL^{-1}) in order to obtain an MOI of 1 and then distributed in 96-well polystyrene plates. Microplates were incubated at 37°C at 120 rpm, and samples collected at 2, 4 and 8 h post infection. The samples were sonicated for 10 s at 30% amplitude, as optimized before, to eliminate cell clusters without affecting cells viability (Freitas *et al.* 2014), and the number of culturable cells (CFU mL^{-1}) was quantified using the microdrop method (Pires *et al.* 2011). Three independent experiments were performed in triplicate. Control experiments were performed by adding SM buffer instead of phage suspension.

Determination of BIM rate

The Bacteriophage Insensitive Mutant (BIM) rate was assessed after 8 h of infection on both exponential and stationary phase cells. The plates were incubated overnight at 37°C. All resulting colonies were counted, and the BIM frequency was determined by dividing the number of surviving colonies by the original bacterial titer (Kaur, Harjai and Chhibber 2014). Moreover, 50 surviving colonies, of each infection, were selected, restreaked three times to guarantee that they were not phage contaminated and then the sensitivity to SEP1 was tested. Using the drop test, SEP1 was placed on each bacterial lawn and, after overnight incubation, the resistance frequency was calculated (number of insensitive out of the 50 selected colonies; 0% means sensitive isolates and 100% means resistant isolates).

Phage one-step growth curve

Phage growth curves on exponential and stationary phase cells were performed to assess phage replication profile. Briefly, 10 mL of a *S. epidermidis* culture was grown for 48 h at 37°C and 120 rpm. Cell suspensions were diluted with the supernatant of the remaining culture (centrifuged media used to grow the cells) to obtain an OD_{600nm} of approximately 0.5 (~5×10^8 CFU mL^{-1}). Regarding exponential phase cells, 10 mL of a *S. epidermidis* culture was grown for at 37°C and 120 rpm, until reach OD_{600nm} of approximately 0.5. An equal volume of phage was added in order to obtain an MOI of 1. Samples were collected for plaque forming unit counts every 15 min until reaching 150 min of infection and plated immediately. Three independent experiments were performed in duplicate.

Gene expression quantification

RNA extraction was performed using phage-infected exponential and stationary phase cells at different time points (5, 10, 15, 30 and 45 min). Cells immediately before phage infection were used as control (t0). qPCR was used to assess the expression of *S. epidermidis* RNA Polymerase (*RNAP*) and SEP1 phage *gp54*, helicase (*hel*), major capsid protein (*mcp*), and endolysin (*lys*). The primers used were designed with the Primer3 software (Rozen and Skaletsky 2000) using the *S. epidermidis* RP62A genome as a template (NC_002976.3) or *S. epidermidis* phage phiIBB-SEP1 (KF021268). In addition, 16S rRNA gene was used as reference gene. The sequences of the primers used are listed in Table 1.

At each time point, total RNA was extracted using the EXTRACTME RNA BACTERIA & YEAST KIT (Blirt, Gdańsk, Poland), using mechanical and chemical lysis, as previously optimized (Franca *et al.* 2012). Contaminating genomic DNA was removed

Table 1. Primer sequences used for gene expression quantification by qPCR.

Gene	Primer sequence (5′ to 3′)	Melting temperature (°C)	Product size (bp)
16S rRNA	F-GGGCTACACACGTGCTACAA	58.24	176
	R-GTACAAGACCCGGGAACGTA	58.36	
RNAP	F-TGTTTGTCGTTTCCGTGGTA	58.46	157
	R-TTGCATGTTTGCTCCCATTA	58.56	
gp54	F-GAAGATGTTGATGAAGGTTTCG	57.24	168
	R-TGAAGCATCTGTATGTTGTAATGC	57.82	
hel	F-CATGGTGAAGTCGATTCTGAG	56.74	150
	R-TGATTTACCTCCTGCCCCTA	58.05	
mcp	F-CGTATCGGTAAAGGCTTTGG	58.18	174
	R-ACGGATAAATCCACGAGCTG	58.60	
lys	F-GCAATGTGGGGAAATGCTAT	58.31	176
	R-TCACCGTAAGGGTCTCCATC	58.38	

by treating total RNA with DNase I (Fermentas, Burlington, ON, Canada) for 30 min at 37°C. The enzyme was then heat-inactivated at 65°C for 10 min in the presence of EDTA. Total RNA was quantified using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and stored at –80°C. Total RNA samples were reverse transcribed using qScript cDNA Synthesis Kit (QuantaBio, Beverly, MA, USA), following manufacturer's instructions. Control reactions lacking the reverse transcriptase enzyme (no-RT) were included. qPCR reactions contained 2 μL of 1:100 diluted cDNA or no-RT control, 1 μL containing 10 pmol of each primer, 2 μL nuclease free deionized H_2O, and 5 μL SsoFast EvaGreen® Supermix (BIO-RAD), with the following thermal cycler parameters: 95°C for 30 s, 40 cycles of 95°C for 5 s and 58°C for 5 s. To monitor the reaction specificity and primer dimer formation, end-products were analyzed by melting curves. A mock qPCR reaction lacking the cDNA template was used. The quantification of mRNA transcripts, for each gene under study, was determined using the Pfaffl method (Pfaffl 2001). Data analysis was based on three independent experiments.

Flow cytometry

Cell viability within exponential and stationary phase phage-infected cells was assessed by flow cytometry as previously optimized (Cerca *et al*. 2011b; Pires and Melo 2018), with minor modifications. In brief, 30 μL of bacterial suspension were added to 270 μL of a solution containing 1:80000 of SYBR green (SYBR—Thermo Fisher Scientific) and 20 μg mL^{-1} propidium iodide (PI—Sigma-Aldrich, St. Louis, MO, USA). A control solution with thermal inactivated phages was added to stationary phase cultures to analyze if the cells responded to other solution components. Samples were taken every 5 min, over a period of 30 min, and then every 15 min until 3 h post-infection. The fluorescence of bacteria was measured using an EC800 (SONY, San Jose, CA, USA) flow cytometer equipped with an argon ion laser emitting a 488 nm. An acquisition protocol was defined to measure green fluorescence (FL1 channel), red fluorescence (FL4 channel), forward scatter (FS) and side scatter (SS). For all detected parameters, amplification was carried out using logarithmic scale. Data were acquired and analyzed using Sony EC800 Flow Cytometry Analyser software (San Jose, CA, USA). Data were also analyzed by FlowJo 10.4.2 trial version software (Tree Star, Ashland, OR, USA). Three independent experiments were performed in triplicate.

Statistical analysis

The assays were compared using two-way ANOVA and Tukey's honest significant difference post hoc test, using Prism 6 (GraphPad, La Jolla, CA, USA). Data are depicted as mean and standard deviation. Differences among conditions were considered statistically significant when $P < 0.01$.

RESULTS

SEP1 reduces the density of stationary phase cultures to a greater extent than exponential phase cultures

Staphylococcus epidermidis phage SEP1 killing efficiency of planktonic cell populations at exponential and stationary phase was tested to understand if host metabolic/physiological state influences phage efficacy (Fig. 1A and B). As expected, SEP1 phages effectively infected exponential phase cells, significantly reducing ($P < 0.01$) the number of culturable cells by 2 orders-of-magnitude after 2 h (Fig. 1A). Phages continued to decrease the number of culturable cells by approximately 5 orders-of-magnitude after 4 h of infection. This cellular concentration was kept constant after 8 h of infection.

Regarding stationary phase cells, barely any reduction was observed during the first 2 h of phage infection (Fig. 1B). However, after 4 h, SEP1 was able to reduce stationary phase cells by approximately 4 orders of magnitude. SEP1 further reduced this bacterial population by up to 7 orders-of-magnitude after 8 h of infection. Curiously, this reduction was higher than the obtained for exponential phase cells ($P < 0.01$).

Exponential cultures have a greater rate of BIMs

It is known that bacterial cells are able to develop mechanisms to resist phage infection (Labrie, Samson and Moineau 2010). Thus, we hypothesized that phage resistance might be responsible for the differences observed in phage killing efficiency of stationary vs exponential planktonic populations. To assess this hypothesis, the rate of development of BIMs after SEP1 challenging was determined both in exponential and stationary phase cultures.

The calculated BIM frequency present in stationary phase cultures was 3.16×10^{-7}, while in exponential cultures this frequency was higher with a value of 1.41×10^{-6}. Resistance frequency for SEP1 was determined by selecting 50 surviving

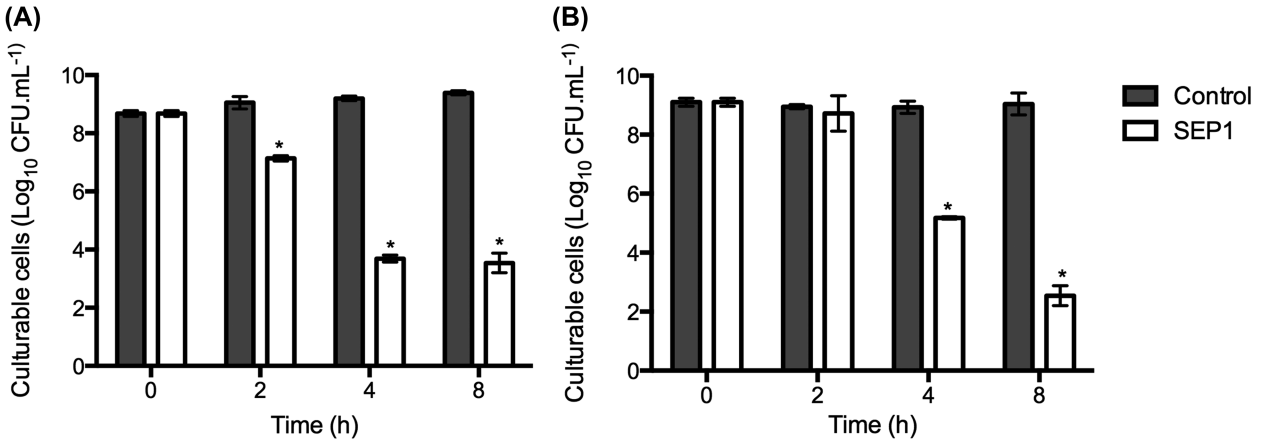

Figure 1. SEP1 phage infection of *S. epidermidis* 9142 planktonic cultures, using a MOI of 1. (**A**) exponential phase cells; (**B**) stationary phase cells. Data were assessed by CFU counting and the values represent the mean plus and minus standard deviation of three independent experiments performed in triplicate. Statistical differences ($P < 0.01$) between control and SEP1-treated cells (*) were determined by two-way repeated-measures analysis of variance (ANOVA) with Tukey's honest significant difference post hoc test.

colonies after 8 h of SEP1 infection of both exponential and stationary phase cultures. After SEP1 infection, 56% of the surviving colonies of exponential phase cells were already resistant to SEP1. In opposition, a lower percentage, 40%, obtained in stationary cultures, showed resistance to SEP1. This result can be in part due to the fact that exponential cultures support the growth of phage insensitive mutants, whereas their growth in stationary cultures might be impaired or decreased.

Stationary phage cells have a low burst size and a longer latency period

Phage interaction with exponential and stationary phase cells was analyzed by determining the infection parameters on these types of cells. Both latent periods and burst sizes were determined based on the results of one-step growth experiments (Fig. 2). On exponential phase cells, SEP1 had a latency period of 45 min and a burst size of 28 PFU per infected cell (Fig. 2). However, when infecting stationary phase cells, SEP1 demonstrated a longer latency period (75 min) and a smaller burst size (7 PFU per infected cell) (Fig. 2).

So far, we have shown that SEP 1 is able to infect stationary cultures causing a high cell reduction with a relatively lower rate of resistance compared to exponential cultures. However, the replication of this phage inside stationary cells is slower and gives a smaller progeny, compared to that obtained in exponential cells. In order to understand how phages interact with both type of cells we followed the phage infection period by flow cytometry.

Flow cytometry assays on stationary infected cultures show an immediate SYBR uptake

The use of LIVE/DEAD fluorochromes to assess bacterial viability by using flow cytometry is particularly useful, as it allows the rapid enumeration of viable bacteria (Berney *et al.* 2007). Although several fluorochromes are available for this purpose, SYBR was used together with PI to discriminate live and dead *S. epidermidis* cells. Moreover, SYBR may also be used as a probe to evaluate the physiological state of *S. epidermidis* cells (Cerca *et al.* 2011b). SYBR stains nucleic acids in both live and dead bacteria, while PI, stains nucleic acids in membrane-compromised bacteria. Since PI has a stronger affinity for DNA, it displaces SYBR from the DNA binding sites, and therefore, dead bacteria fluoresce red in contrast to viable bacteria that fluoresce green. Figure 3 depicts the bacterial populations after being challenged with SEP1. On exponential phase cells, it is possible to observe that there were no significant changes in the SYBR mean fluorescence intensity (MFI) between the first 60 min of infection, with an average MFI of 304 ± 38 (Fig. 3A). After 150 min, there was a significant decrease of SYBR MFI, an increase of injured cells (double-positive for SYBR and PI) and an increase of cell debris (non-fluorescent events) which might be associated with a decrease of total cell counts (Fig. S1a, Supporting Information).

On stationary phase cells, an increase on SYBR MFI on the majority of the bacterial population immediately after phage addition was observed (Fig. 3B), whereas a minor cell population showed a decrease on SYBR MFI. During the first 30 min post-phage infection, there was a continuous increase of SYBR MFI 146± 41 to 281 ± 23 (Fig. 3B). The SYBR fluorescence intensity was maintained from 30 to 60 min after which the MFI decreased. At 150 min, an increase of injured cells and cellular debris associated with phage-induced cell lysis were also observed (Fig. S1b, Supporting Information). Control experiments on stationary phase cells demonstrated that cells did not respond to an inactivated phage solution, with stable levels of SYBR MFI during 60 min (Fig. S2, Supporting Information).

After observing the quick response of stationary phase cells to phage addition, quantitative PCR (qPRC) experiments were performed to assess if in these first minutes of contact with phage, cells were already expressing phage genes.

The level of SEP1 early genes' expression increases immediately after phage challenging of stationary cultures

The expression levels of bacterial RNA polymerase (RNAP) and phage *gp54*, helicase (*hel*), major capsid protein (*mcp*) and endolysin (*lys*) genes were assessed at 0, 5, 10, 15, 30 and 45 min post-infection of exponential and stationary phase cells (Fig. 4).

While on exponential phase cells, there were no significant changes on the levels of RNAP transcripts during the first 45 min of infection ($P > 0.01$), on stationary phase cells the level of RNAP

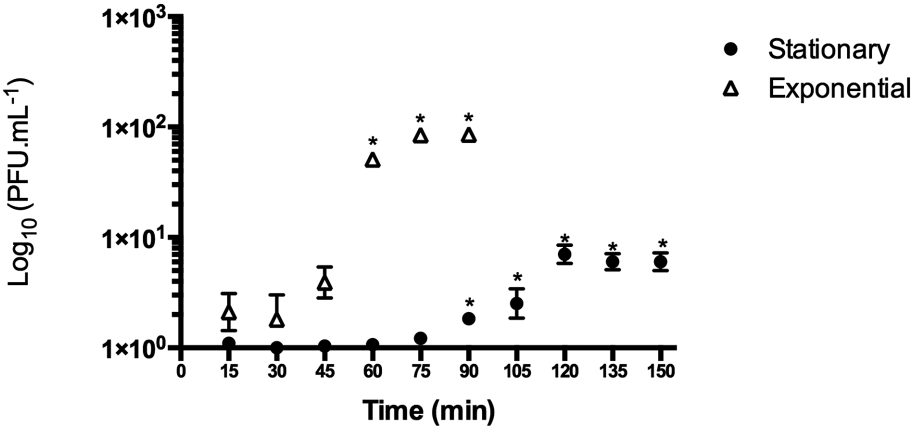

Figure 2. One-step growth curves of SEP1 phage in *S. epidermidis* 9142 exponential phase cells and stationary phase cells. Data points represent the mean plus or minus standard deviation of three independent experiments. Statistical differences ($*P < 0.01$) were analyzed with ANOVA with Tukey's honest significant difference post hoc test.

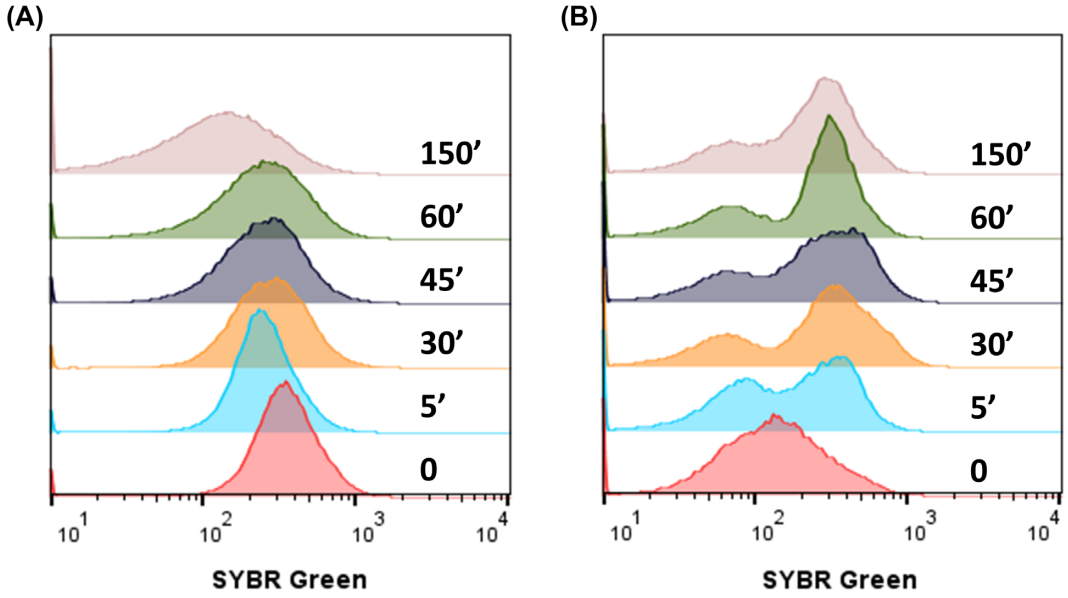

Figure 3. Flow cytometric analysis of *S. epidermidis* 9142 cells, infected with SEP1, using a MOI of 1. Histogram overlay showing SYBR MFI of (**A**) exponential and (**B**) stationary phase cells, prior (0 min) and after (5, 30, 45, 60 and 150 min) SEP1 phage infection. Results are a representative example of three independent experiments.

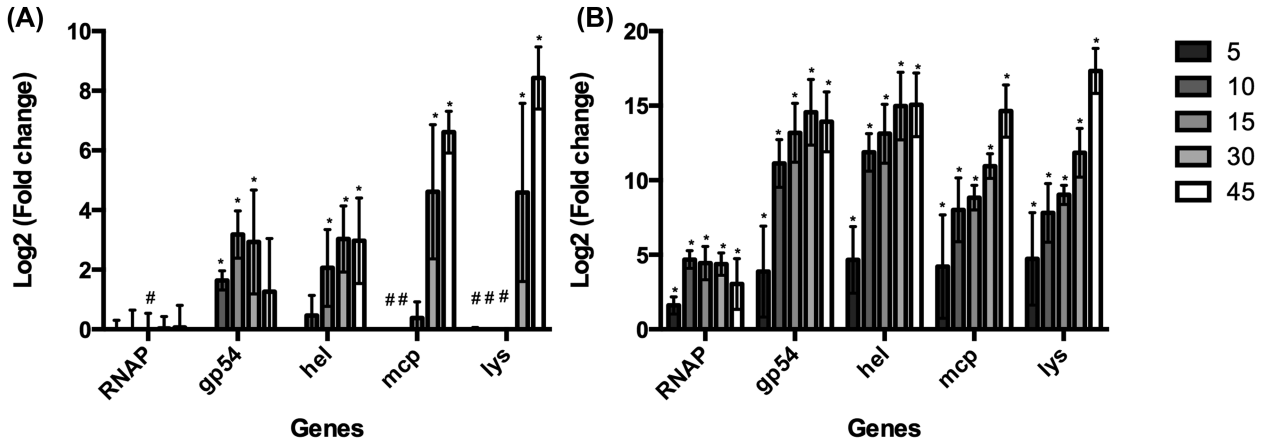

Figure 4. *Staphylococcus epidermidis* 9142 cells gene expression after phage infection. (**A**) on exponential phase cells; (**B**) on stationary phase cells. The quantification of mRNA transcripts was determined using a variation of the Livak method ($E^{\Delta Ct}$), with 16S rRNA as reference gene and non-infected cells as a control. The values represent the mean plus or minus standard deviation of three independent experiments performed in triplicate. Differences between −0.5 and 0.5 Log_2 fold change are marked with #. Statistical differences ($*P < 0.01$) were analyzed using ANOVA with Tukey's honest significant difference post hoc test.

increased slightly 5 min after phage addition and significantly (4-fold) after 10 min. These levels were maintained throughout the first 45 min of phage infection.

Unsurprisingly, the levels of *gp54* increased significantly during the first 5 min of infection ($P < 0.01$) of exponential phase cells. Although not statistically significantly, it was also possible to detect an increase on the levels of *hel* transcripts ($P > 0.01$). *Hel* and *gp54* levels continue to increase until the 30 min of infection (approximately 3-fold) ($P < 0.01$). Simultaneously, only at this time point it was possible to observe an overexpression of about 4.5-fold of *mcp* and *lys* genes ($P < 0.01$). The expression levels of the four phage genes kept stable in the 45 min time point ($P < 0.01$).

Regarding stationary phase cells, it was observed that all four selected genes had a 4-fold increase on their expression just 5 min post phage infection ($P < 0.01$). Moreover, the transcripts levels significantly increased throughout the first 30 min of phage infection ($P < 0.01$). Although the expression levels of *lys* and *mcp* continued to increase after 45 min of infection, the levels of the *gp54* and *hel* stabilized at this time point.

DISCUSSION

The interest in alternatives to antibiotic-based therapies for the treatment of antibiotic-resistant infections has increased in the last decades, with phages re-emerging as promising tools to combat these infections (Harper, Anderson and Enright 2011). Although there are several reports on the efficacy of phages against planktonic cells in the exponential growth phase, few studies have focused on their use against stationary phase cells (Robb, Robb and Woods 1980; Sillankorva *et al*. 2004a; Cerca, Oliveira and Azeredo 2007; Pires *et al*. 2011; Gutierrez *et al*. 2014). Previous reports, using a diversity of phage-host systems, have described a reduced efficacy of phages infecting cells near or at the stationary phase (Ricciuti 1972; Middelboe 2000; Sillankorva *et al*. 2004b). The poor infectivity of phages in stationary phase cells has been mainly described as a consequence of the fewer phage-adsorption sites available, the thicker cell wall and lower metabolic activity in this type of cells. Moreover, Weitz and Dushoff (2008) reported that phage infection of stationary phase cells increased the number of non-viable infections. We have previously shown that SEP1 has a broad host range (Melo *et al*. 2014) and caused a quick and efficient infection of exponential phase cells. In this study, *S. epidermidis* stationary phase cells were prepared by adjusting optical density with spent media that not only results in nutrient depletion, but also introduce some extracellular compounds present in this late growth phase (Bayston and Rodgers 1990). It was therefore surprising to observe that, although the infection of stationary phase cells with SEP1 was delayed for 4 h, SEP1 caused a higher cellular lysis in stationary than in exponential cultures, after 8 h of contact. The fact that no significant reductions were observed during the first 2 h of infection, might be explained by the longer latency period and smaller burst size observed in stationary phase cells (Fig. 2). Similar results were reported in *Escherichia coli* and *Salmonella* spp. (Hadas *et al*. 1997; Santos *et al*. 2014). The higher BIM rate in exponential phase cells might be a consequence of the selection of beneficial mutations that confer resistance to SEP1 that are less likely to occur in non-dividing cells. Despite, the percentage of resistance detected on the selected colonies, the BIM rates are in line with those observed for *S. aureus* phage MR-5 (Kaur, Harjai and Chhibber 2014). It was also reported, in *P. aeruginosa* phage infection experiments, a similar regrowth event associated with the presence of resistant phenotypes after 8 h of infection (Pires *et al*. 2011). The effect of SEP1 against stationary phase cells is therefore of extreme relevance, since phages can target this population that many antibiotics fail to address.

Flow cytometry analysis, using cell viability fluorochromes, was performed to understand SEP1 interaction with exponential and stationary phase cells, as previously reported (Pires and Melo 2018). While, no significant changes on SYBR MFI on exponential phase populations were observed during the first 30 min of infection, on stationary phase cells there was a constant increase of SYBR MFI within this time period, suggesting an increase of metabolic activity (Cerca *et al*. 2011a; Cerca *et al*. 2014). One of the main characteristics of Kayviruses is their short latency period (Vandersteegen *et al*. 2011), after which phage production inside the cells occurs. In our study, the increase of SYBR MFI detected on stationary phase cells might be due to an increase of mRNA as a result of the production of new phage particles. In opposition, as exponential phase cells are highly active it is not expected that phage infection leads to an increase of mRNA transcripts.

As a member of the *Spounavirinae* family, SEP1 does not encode its own RNAP (O'Flaherty *et al*. 2004; Stewart *et al*. 2009; Lobocka *et al*. 2012), which is indicative that this phage might depend on the host RNAP to replicate. Our gene expression results corroborate the flow cytometry results. While on exponential phase cells there was not an overexpression of host RNAP, on stationary phase cells there was an evident overexpression of host RNAP after phage addition, which was constantly overexpressed during the phage replication cycle. This indicates that SEP1 can efficiently hijack the host transcription machinery of stationary phase cells to complete its infectious cycle. Similar results were obtained with *P. aeruginosa* phages PAK_P3 and PAK_P4 (Blasdel *et al*. 2017).

Regarding the expression of phage genes, just after 5 min of contact with phages, both exponential and stationary phase cells were already expressing some of the selected genes. Phage genes are usually divided into early, middle and late genes based on their order of expression in phage development (Pavlova *et al*. 2012). In order to evaluate all the phases of phage infection, the expression of early (*gp54*), middle (*hel*) and late (*lys* and *mcp*) associated genes was determined. On exponential phase cells, *gp54* was the only gene being overexpressed after 5 min. However, on stationary phase cells at this time point the expression of this gene was similar to the other three genes tested. Based in our results, we can speculate that early genes are expressed earlier in stationary phase cells. Middle genes, such as *hel* gene, are mostly involved in DNA replication-associated functions. The fact that *hel* expression levels increased constantly during the first 30 min of infection suggested that phage DNA was being replicated in *S. epidermidis* cells. Late genes are usually genes associated with lysis, DNA packaging or morphogenetic functions. In that sense, *mcp* and *lys* were included in this analysis. Although the transcription of *gp54* and *hel* was stabilized after 30 min of contact with cells, there was still an increase on the expression levels of *mcp* and *lys* genes after 45 min on both exponential and stationary phase cells. These results emphasize the modular feature of phage genomes, in which there are several transcriptional units during their infection (Leskinen *et al*. 2016). In SEP1s related *Bacillus* phage SPO1, 58 transcriptional units were proposed (Stewart *et al*. 2009). Moreover, the presence of numerous putative promoters and rho-independent terminators in SEP1 genome emphasizes this complex transcriptional organization (Vandersteegen *et al*. 2011; Melo *et al*. 2014).

The fact that non-replicating cells, such as the stationary phase, responded promptly to phage addition, contrary to what was observed previously in *E. coli* starved cells infected with T4 phage (Bryan *et al.* 2016), broadens new horizons of phage-host interactions.

CONCLUDING REMARKS

The phenomenon of SEP1 infecting efficiently planktonic cells with different physiological states, suggests that this phage might be a good candidate for being a component of a therapeutic phage cocktail. Although the replication time is longer in stationary cultures, both transcriptional and flow cytometry assays demonstrated that SEP1 can initiate its lytic cycle immediately after entering in contact with stationary-phase cells. A whole-transcriptomics analysis from both phage and host point of view and the analysis of the activity of this phage against more complex communities, namely biofilms, would be necessary to better understand the mechanisms responsible for infecting so efficiently stationary phase.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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Conflicts of interest. None declared.

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