

# A standardized protocol for the UV induction of *Sulfolobus* spindle-shaped virus 1

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**Abstract** The *Fuselloviridae* prototype member *Sulfolobus* spindle-shaped virus 1 is a model of UV-inducible viruses infecting *Crenarchaeota*. Previous works on SSV1 UV induction were based on empirically determined parameters that have not yet been standardized. Thus, in many peer reviewed literature, it is not clear how the fluence and irradiance have been determined. Here, we describe a protocol for the UV induction of SSV1 replication, which is based on the combination of the following instrumentally monitored parameters: (1) the fluence; (2) the irradiance; (3) the exposure time, and (4) the exposure distance. With the aim of finding a good balance between the viral replication induction and the host cells viability, UV-irradiated cultures were monitored for their ability to recover in the aftermath of the UV exposure. This UV irradiation procedure has been set up using the well-characterized *Sulfolobus solfataricus* P2 strain as model system to study host–virus interaction.

**Keywords** *Sulfolobus* spindle-shaped virus · UV induction · Irradiation protocol · *Fuselloviridae* · Viral titre · Plaque assay · Fluence · Irradiance

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## Introduction

*Sulfolobus* spindle-shaped virus 1 (SSV1), isolated from the native host *Sulfolobus shibatae* in Beppu (Japan), is the prototype and the best characterized member of the *Fuselloviridae* family (Prangishvili 2013; Martin et al. 1984). SSV1 can propagate only in few hosts (Ceballos et al. 2012), among which a strain of *S. solfataricus* isolated from the solfataric field of Pisciarelli near Naples (Italy), turned out to be suitable for carrying out genetic (Stedman et al. 1999; Clore and Stedman 2007; Iverson and Stedman 2012), biochemical (Kraft et al. 2004a, b; Menon et al. 2008; Zhan et al. 2012; Eilers et al. 2012) and physiological studies (Reiter et al. 1987; Schleper et al. 1992; Zillig et al. 1980; Fröls et al. 2007a; Fusco et al. 2013).

The genome of SSV1 is a double-stranded DNA molecule of 15 Kbp, which has been found both as integrated (provirus) and as episomal form into the host cells (Schleper et al. 1992; Yeats et al. 1982). Its complete sequence has been determined (Palm et al. 1991), and as for other fuselloviruses (Stedman et al. 2003; Wiedenheft et al. 2004; Redder et al. 2009; Contursi et al. 2007, 2010, 2014a), it encodes for a number of quasi-orphan proteins, which do not have detectable homologues in the databases other than in related hyperthermophilic viral genomes (Contursi et al. 2013). This has led to the necessity of performing structural and functional analyses to unravel their functions. For instance, the structure of several SSV1 transcription factors (TFs) has been solved revealing that, despite the lack of homology, most of these viral TFs are bacterial like (Kraft et al. 2004a, b; Menon et al. 2008; Zhan et al. 2012; Eilers et al. 2012; Contursi et al. 2013; 2011; 2014b).

Insights about the role of some SSV1 proteins have been derived from genetic analyses that revealed some of the

essential SSV1 genes (Stedman et al. 1999; Iverson and Stedman 2012). Furthermore, its genome has been used as template for the construction of replicative and expression vectors, which have been employed for heterologous gene expression as well as for studying viral ORFs essentiality (Contursi et al. 2014a; Stedman et al. 1999; Iverson and Stedman 2012; Jonuscheit et al. 2003; Albers et al. 2006; Cannio et al. 1998; Cannio et al. 2001).

Pioneering studies on SSV1 helped shedding light on how gene expression is regulated in *Archaea*. In particular, the elucidation of the SSV1 transcriptional map as well as the identification of all the transcriptional start sites (TSSs) (Reiter et al. 1987; Fröls et al. 2007a; Fusco et al. 2013), led to the discovery of two conserved sequence motifs that resemble those of the eukaryotic basal gene promoters recognized by the RNA polymerase II (Reiter et al. 1988). Therefore, the bacterial-like transcription regulators encoded by *Archaea* operate in a eukaryal-like transcriptional context (Contursi et al. 2013).

Upon infection, one copy of the viral genome site specifically integrates into the host chromosome at an arginyl-tRNA gene (Schleper et al. 1992), whereas the episomal form (~5 copies per cell) is maintained in host cells in three isoforms, i.e. (1) as positively or negatively supercoiled and (2) as relaxed double-stranded DNA (Snyder et al. 2003). Intriguingly, so far SSV1 is the only member of the *Fusell-oviridae* family that shows an UV-inducible life cycle. Upon UV light exposure a well-characterized gene expression pattern is triggered and involves the expression of a short UV-inducible transcript, namely  $T_{ind}$ , followed by: (1) the time-coordinated expression of all the other viral transcripts, (2) the induction of the SSV1 genome replication (Fröls et al. 2007a) and (3) a steep increase of the viral titre. Only recently efforts have been made to get insights into the molecular switch from the lysogenic state to the replication induction (Fusco et al. 2013). Nevertheless, mechanisms underpinning these processes are still murky.

So far, in peer reviewed published literature, it is not clear how the fluence (or UV dose) and the irradiance have been measured. Indeed, the fluence ( $J m^{-2}$ ) administered to the cells has been only empirically determined and no attention has been paid to monitoring the irradiance ( $J m^{-2} s^{-1}$ ) (Martin et al. 1984; Reiter et al. 1987; Schleper et al. 1992; Fröls et al. 2007a). Furthermore, the negative effect on the host viability, that the UV treatment implies, has been underestimated. Since an essential parameter such as the irradiance has not been taken into account elsewhere (Martin et al. 1984; Reiter et al. 1987; Schleper et al. 1992; Fröls et al. 2007a) and the nomenclature may be misleading, important definitions need to be discussed. The irradiance is a proper term used when a surface is irradiated by UV light coming from all directions above the aforementioned surface. Indeed, the irradiance is the total radiant power incident from

all upward directions on an infinitesimal element of surface having area  $dA$  and containing the point under consideration divided by  $dA$ , as defined by Bolton (Bolton and Linden 2003). When the irradiance is constant (as herein described), the fluence is derived multiplying the irradiance by the exposure time (in seconds). The term UV dose, so far used to refer to the fluence, is confusing since the word “dose” describes a total energy adsorbed by a surface (e.g. the skin). In UV irradiation of microbial suspensions, the major amount of the incident UV light crosses the sample with only a small percentage being adsorbed by the cells. For this reason, fluence is a much more appropriated term since it is related to the incident UV energy, rather than to the adsorbed fraction (Bolton and Linden 2003).

Herein, we describe a protocol that has been developed with the purpose of: (1) standardizing all the parameters needed for performing an UV induction experiment on SSV1 lysogens and (2) finding a good balance between the viral induction and the host viability. Indeed, we show that by tuning fluence and irradiance, cells viability can be improved and, in turn, the viral induction reaches highest values determined so far.

## Materials and methods

### Growth conditions and UV irradiation

Cells of the *S. solfataricus* P2 lysogenic strain (SSV1-P2) were revitalized by depositing few microliters of culture onto the soft layer of an SCVYU-Gelrite plate and incubating at 75 °C for 3–5 days, as described elsewhere (Contursi et al. 2006). Subsequently, local growth areas (spots) were inoculated into 50 ml of SCVY medium, i.e. a glycine-buffered Brock’s basal salt solution, supplemented with 0.2 % sucrose (wt/vol), 0.2 % casamino acids (wt/vol), 1 × vitamins (Wolin et al. 1963) and 0.005 % yeast extract (wt/vol); the pH was adjusted to 3.5 with concentrated  $H_2SO_4$ . Cells cultivation was conducted in a 250-ml Erlenmeyer flask with a long neck, at 75 °C with a shaking rate of 180 rpm using a MaxQ™ 4000 Benchtop Orbital Shaker (Thermo Scientific). The cell growth was spectrophotometrically monitored at 600 nm ( $OD_{600}$ ) by means of a Varian Cary® 50 Bio UV/Visible Spectrophotometer (McKinley Scientific). Once the culture reached the logarithmic phase of growth (0.4–0.6  $OD_{600}$ ), it was diluted to a value of 0.05  $OD_{600}$  in 50 ml of fresh SCVY medium and let to grow up to 0.8  $OD_{600}$ .

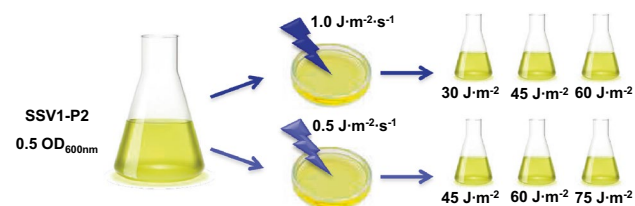
Before performing UV irradiations, the SSV1-P2 culture was diluted in 400 ml of SCVY medium to 0.08  $OD_{600}$  into a 1-L Erlenmeyer flask and let to grow up to the mid-logarithmic growth phase (0.5  $OD_{600}$ ). Aliquots of this culture were then UV-irradiated or mock treated. In detail, 40 ml of

culture was transferred into a 150 × 25-mm Petri plate (BD Falcon™) and UV irradiation was carried out at room temperature in a dark room under red light, by carefully hand shaking the plate under a germicidal lamp G15T8 (254 nm, 15 W, Sankyo Denki). Differently from previous reports (Martin et al. 1984; Reiter et al. 1987; Schleper et al. 1992; Fröls et al. 2007a), the fluence and the irradiance were instrumentally measured by means of a Quantum-photo-radiometer HD9021, equipped with an LP9021 UVC probe (Delta Ohm). The fluence was of 30, 45 and 60 J m<sup>-2</sup> (with an irradiance of 1.0 J m<sup>-2</sup> s<sup>-1</sup>) or of 45, 60 and 75 J m<sup>-2</sup> (with an irradiance of 0.5 J m<sup>-2</sup> s<sup>-1</sup>) (Fig. 1). The irradiance was tuned by changing the exposure distance, which was of 70 cm (for an irradiance of 0.5 J m<sup>-2</sup> s<sup>-1</sup>) or of 50 cm (for an irradiance of 1.0 J m<sup>-2</sup> s<sup>-1</sup>), whereas the desired fluence was achieved by changing the exposure time to the UV light source. As control, a mock-treated sample was subjected to the same procedure except for the UV light exposure. Treated samples were separately collected in 250-ml Erlenmeyer flasks, which were wrapped with aluminium foil to protect the culture from further light exposure, and incubated at 75 °C with a shaking rate of 180 rpm.

To check cells viability immediately after the treatment, serial dilutions of the cultures were plated on SCVYU-Gelrite and incubated at 75 °C for 7–10 days. Colonies were counted (100–300 cells per plate) and a survival percentage was calculated. Moreover, cell growth was spectrophotometrically monitored throughout the post-treatment incubation and samples were taken after 8 and 24 h, because a peak in the amount of SSV1 DNA and in the viral titre was expected, respectively (our unpublished data). Cellular pellets as well as cell-free supernatants were obtained through centrifugation at 3,000×g for 15 min using the Centrifuge 5810R (Eppendorf). The procedure was carried out in triplicate and average (Avg) as well as standard deviation (SD) were calculated for the data reported below.

### Quantitative plaque assay

SSV1 viral titre was determined for cell-free supernatants by quantitative plaque assays using the uninfected *S.*



**Fig. 1** Schematic illustration of the UV irradiation procedure. Cells were grown exponentially until 0.5 OD<sub>600</sub> before being irradiated with an irradiance of 0.5 or 1.0 J m<sup>-2</sup> s<sup>-1</sup>

*solfatarius* strain P2, as lawn. This strain was first revitalized on SCVYU-plate and then transferred into 50 ml of SCVYU medium, as described above. Cell density was monitored spectrophotometrically at 600 nm until the late logarithmic phase of growth (0.6 OD<sub>600</sub>).

Lower layers of SCVY-Gelrite were prepared by pouring, in 100 × 15 mm plastic plates (Falcon), 30 ml of 1 × SCVY mixed with Gelrite® (Sigma Aldrich) at the final concentration of 0.8 % (w/v). Subsequently, 100 μl of serial dilutions (from 10<sup>-4</sup> to 10<sup>-8</sup>) of cell-free supernatants (containing SSV1 viral particles) were added to a mix composed of: (1) 1 × SCVY medium, (2) Gelrite® at the final concentration of 0.4 % (w/v) and (3) 0.5 ml of the 0.6 OD<sub>600</sub> *S. solfatarius* P2 culture (about 0.5 × 10<sup>8</sup> cells). Each mix (the upper layer) was poured onto the lower layer of a pre-warmed SCVY-Gelrite plate. After a short incubation at room temperature to allow gelification of the upper layers, plates were transferred to 75 °C for 5–7 days. Growth inhibition areas (turbid halos), which appeared onto the upper layer as consequence of local growth retardation, were counted (up to 100 plaques per plate) and the viral titre (PFU/ml) was calculated considering the dilution factor.

Although quantitative plaque assay of SSV1 viral particles is notoriously challenging, we have noticed that a critical point for obtaining clearer halos depends on the physiological state as well as on the number of the cells used as lawn. Indeed, when about 0.5 × 10<sup>8</sup> cells of a not-freshly diluted culture are used, clear halos appear on the plate surface upon infection (Supplementary material, S1). Conversely, if the culture is freshly diluted with pre-warmed medium before plating, halos appear turbid and difficult to be counted.

### Semi-quantitative PCR analysis

SSV1-P2 pellets, collected 8 h post-irradiation, were treated for total DNA extraction using the DNeasy tissue kit (Qiagen), following the manufacturer's instructions. The concentration of the DNA samples was spectrophotometrically measured by means of a Nanodrop 2000 Spectrophotometer (Thermo Scientific). To detect variations of the viral DNA content, total DNA samples from mock-treated and UV-irradiated cells were analysed by semi-quantitative PCR assays. With this aim, two primer couples were designed (Fusco et al. 2013) using Primer3 software (available at the website: <http://bioinfo.ut.ee/primer3-0.4.0/>), to amplify: (1) a 155-bp region of the SSV1 single-copy gene *vp2* and (2) a 108-bp region of the host single-copy gene *orc1* (Table 1). A PCR master mix was prepared as follows: 1 × Taq buffer, 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP mix (Thermo Scientific), 0.6 μM *orc1*-fw, 0.6 μM *orc1*-rv, 0.6 μM *vp2*-fw, 0.6 μM *vp2*-rv and 0.1U/μl of Taq DNA Polymerase (Thermo Scientific). Aliquots of the master mix (60 μl each) were dispensed into 200-μl tubes (Eppendorf) and 100 ng of total DNA from

**Table 1** Sequences of oligonucleotides used for the semi-quantitative PCR assays

Name	Sequence (5'-3')	Length (nt)
<i>orc1-fw</i>	TATAAATTGTTATAGACATAGAACGCTGTA	30
<i>orc1-rv</i>	TTAAATACTTCTTGTGCCGATAGTCC	26
<i>vp2-fw</i>	GGAGGGTACATCGCTACCTTATGA	24
<i>vp2-rv</i>	CAGTAGGGCTGACAGTAAACTACG	24

mock-treated or UV-irradiated cells were added. Each aliquot was then split into three sub-aliquots (20  $\mu$ l each), to collect the tubes at the 20th, 25th and 30th cycle of amplification. The thermal cycling protocol was carried out into a Mastercycler Personal (Eppendorf®) as follows: an initial denaturation step of 5 min at 95 °C, followed by 30 cycles of 40 s at 95 °C, 40 s at 62 °C, and 1 min at 72 °C. After collecting the samples at the 30th cycle, a final step at 72 °C has been carried out for 10 min. PCR products were run on a 2 % agarose gel in 1  $\times$  TAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM EDTA) for 1 h. Pictures were taken by means of a Gel Doc XR System (Biorad) and DNA bands quantified using the Quantity One Software (Biorad).

## Results

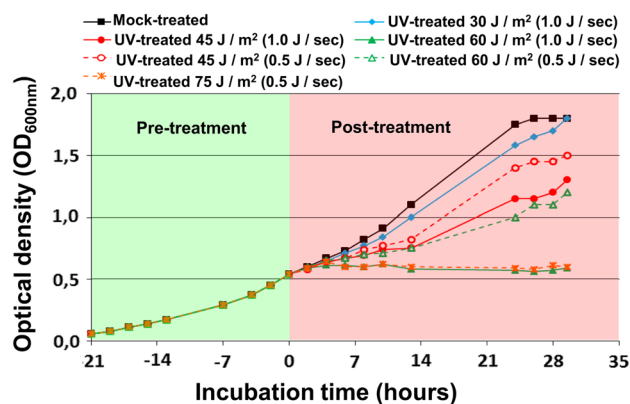
The UV irradiation has a dose-dependent effect on the host cell viability

The UV irradiation not only induces the viral replication in SSV1-lysogens, but affects metabolism and survival of the host as well. In fact, major changes in growth rate and gene expression have been observed in the aftermath of UV irradiation of *S. solfataricus* cells.

Nevertheless, discrepant data have been produced about the UV-dependent gene regulation in this crenarchaeon, probably because the equipment and the procedure used, have not been standardized (Fröls et al. 2007b, 2009; Götz et al. 2007; Salerno et al. 2003).

Likewise most of the bacteriophages and viruses known, the SSV1 development seems to be sensitive to the physiological state of the host (Prangishvili 2013; Contursi et al. 2006; Bondy-Denomy and Davidson 2014). Therefore, a suitable protocol for SSV1 induction requires taking into account the host response in regard of cell viability and ability to recover upon UV exposure.

With the aim of setting up the best conditions for the induction of the SSV1 replication, cells were UV-irradiated by combining a set of different parameters: (1) the fluence ranging from 30 to 75  $\text{J m}^{-2}$ , (2) the irradiance of 0.5 or 1.0  $\text{J m}^{-2} \text{s}^{-1}$ ; (3) the exposure times ranging from 30 to 150 s, and (4) the exposure distance of 50 or 70 cm.



**Fig. 2** Growth curves of SSV1-P2 pre- and post-UV treatment. The  $\text{OD}_{600\text{nm}}$  values were measured over a time window of  $\sim 55$  h. Cells were grown exponentially until 0.5  $\text{OD}_{600\text{nm}}$  value (21st h of incubation) before being mock or UV treated (green area of the graph). Afterwards, the samples were split into seven flasks, incubated back to 75 °C and further monitored (red area of the graph). The growth retardation is related to the fluence and irradiance administered

As shown in Fig. 2, cell growth is slowed down by the treatment with a dose-dependent trend. The highest UV fluence levels, i.e. 60  $\text{J m}^{-2}$  and 75  $\text{J m}^{-2}$ , resulted in heavy growth retardation, probably as consequence of an impaired activation of the DNA lesions repairing system(s). Conversely, cell growth was only partially delayed when the fluence and irradiance were progressively reduced down (Fig. 2). Furthermore, cells viability determined by plating aliquots of the cultures, revealed that the survival percentage upon UV irradiation gradually increased by reducing both the fluence and irradiance (Table 2). To compensate for effects related to temperature changes, control cultures were subjected to the same procedure except for the UV irradiation.

Interestingly, our data indicate that the fluence ( $\text{J m}^{-2}$ ) is not the only parameter affecting the cell survival (Table 2) and their ability to recover after the treatment (Fig. 1). Indeed, the irradiance ( $\text{J m}^{-2} \text{s}^{-1}$ ) is crucial to preserve cells viability. In fact, using a milder irradiance reduces cells lethality and, in turn, improves the viral replication (see below), which relies on the host machinery. Indeed, the same fluence provided with two values of irradiance (0.5 or 1.0  $\text{J m}^{-2} \text{s}^{-1}$ ) led to different percentage of viability. For all the values of fluence tested, the percentage of survival cells is higher when they are treated with a lower irradiance (0.5  $\text{J m}^{-2} \text{s}^{-1}$ ; Table 2).

A suitable UV fluence for the induction of the SSV1 replication

The effect of UV irradiation on the SSV1 replication has been evaluated by monitoring the relative amount of the SSV1 DNA in the mock-treated and UV-irradiated cultures



**Table 2** Cells viability after UV treatment

Total fluence (J m <sup>-2</sup> )	CFU/ml Avg ± SD (n = 3)	Survival percentage (%) <sup>a</sup>
0	8.63 × 10 <sup>7</sup> ± 0.26 × 10 <sup>7</sup>	100
30 <sup>b</sup>	6.07 × 10 <sup>7</sup> ± 0.30 × 10 <sup>7</sup>	70.33
45 <sup>c</sup>	3.17 × 10 <sup>7</sup> ± 0.08 × 10 <sup>7</sup>	36.73
45 <sup>b</sup>	1.87 × 10 <sup>7</sup> ± 0.08 × 10 <sup>7</sup>	21.67
60 <sup>c</sup>	0.60 × 10 <sup>7</sup> ± 0.06 × 10 <sup>7</sup>	6.95
60 <sup>b</sup>	0.23 × 10 <sup>7</sup> ± 0.02 × 10 <sup>7</sup>	2.67
75 <sup>c</sup>	0.51 × 10 <sup>6</sup> ± 0.01 × 10 <sup>6</sup>	0.59

<sup>a</sup> Survival percentages calculated considering the mock-treated sample as 100 %

<sup>b</sup> Cells irradiated with an irradiance of 1.0 J m<sup>-2</sup> s<sup>-1</sup>

<sup>c</sup> Cells irradiated with an irradiance of 0.5 J m<sup>-2</sup> s<sup>-1</sup>

by semi-quantitative PCR assays. Cells were irradiated with a set of different conditions as described above and collected 8 h post-irradiation. Two single-copy genes on the host and viral chromosomes (*orc1* and *vp2*, respectively) were chosen to provide an estimation of the viral genome content under all the conditions tested. As shown, the PCR products were analysed for each sample on agarose gel at the 20th, 25th and 30th cycle of amplification (Fig. 3).

Densitometric analysis, performed by means of the software Quantity One (BioRad), revealed that the *vp2/orc1* fluorescence ratio increases in all the UV-irradiated samples compared to the mock-treated ones (when the same amplification cycle is considered). Notably, the highest value was detected for the sample treated with an irradiance of 0.5 J m<sup>-2</sup> s<sup>-1</sup> and a fluence of 45 J m<sup>-2</sup>, which, therefore, is the most suitable condition among the several tested (Fig. 3). For this latter sample, the fluorescence intensity of *vp2* at the 20th amplification cycle is comparable to its intensity at the 25th amplification cycle of the mock-treated sample (lysogenic culture). Under the best conditions, at each amplification cycle the amount of a specific amplicon

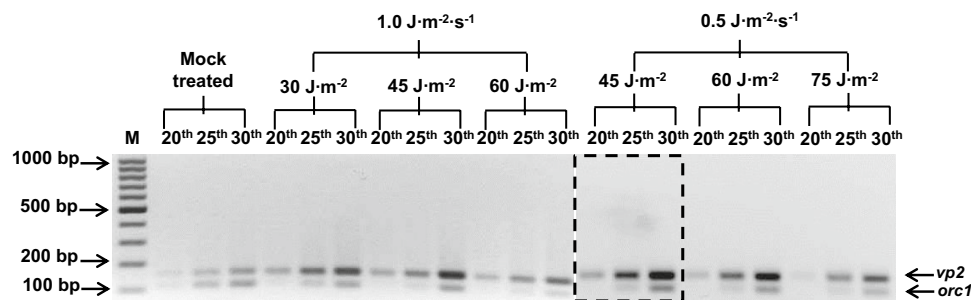
increases by a factor of 2<sup>n</sup>, where *n* is the number of cycle. Since the same amount of *vp2* (fluorescence intensity) is reached 5 cycles in advance in the irradiated sample, the copy number of SSV1 in this latter is ~32-fold higher (2<sup>5</sup>) than in the mock-treated one. Being the SSV1 copy number ~5 episomes per cell, the total viral amount reaches 160 copies per cell.

Worth of note is that, using the QIAprep Spin Miniprep Kit (Qiagen), about 20 μg of SSV1 DNA (~1.2 × 10<sup>12</sup> SSV1 episomal genomes) were isolated from independent preparations, by processing 50-ml pellets of cells harvested 8 h post-irradiation (0.75 OD<sub>600</sub>). If it is assumed that 1 OD<sub>600</sub> culture contains about 2 × 10<sup>8</sup> cells/ml (as calculated by plate efficiency), 50 ml of a 0.75 OD<sub>600</sub> culture contains ~7.5 × 10<sup>9</sup> cells. According to the data already measured by the densitometric analysis, the initial SSV1 copy number increases of 32-fold.

The SSV1 viral particles accumulate in the culture supernatant after the irradiation

Performing quantitative plaque assay to determine the SSV1 viral titre is notoriously challenging. However, we have noticed that using a not-freshly diluted culture as lawn, the resulted halos appeared clearer and easier to be counted than those obtained when a freshly diluted culture was plated. Therefore, the growth retardation induced by SSV1 infection is more pronounced when the culture is harvested from an exhausted medium.

Viral titre was determined for the culture supernatants harvested at the 8th and the 24th h post-irradiation. These two time points were chosen because after 8 h of incubation the amount of viral DNA in the cells reaches its maximum, while subsequently decreases (10–24 h), probably as consequence of the viral particles extrusion into the culture medium (our unpublished data). The highest amount of viral particles (5 × 10<sup>9</sup> PFU/ml) was produced from cells treated with a fluence of 45 J m<sup>-2</sup> (irradiance of



**Fig. 3** Semi-quantitative PCR analysis on DNA from mock- and UV-treated SSV1 -P2 cultures. Agarose gel electrophoresis of PCR products collected at the 20th, 25th and 30th amplification cycle. Black-straight arrows point out to molecular weight markers as well as to host

(*orc1* = 108 bp) and viral (*vp2* = 155 bp) PCR products. Densitometric analysis detected a maximum amount of SSV1 DNA for the sample irradiated with a fluence of 45 J m<sup>-2</sup> and an irradiance of 0.5 J m<sup>-2</sup> s<sup>-1</sup> (dashed frame)

$0.5 \text{ J m}^{-2} \text{ s}^{-1}$ ) and harvested 24 h post-irradiation. Whilst, the same fluence ( $45 \text{ J m}^{-2}$ ), provided using an irradiance of  $1.0 \text{ J m}^{-2} \text{ s}^{-1}$ , led to a lower viral titre ( $6 \times 10^8$  PFU/ml). This difference is due to the fact that, in the former case, a larger fraction of the cell population is viable (37 vs 22 %, Table 2), thus better supporting viral replication and virions extrusion.

## Discussion

Three decades have passed since the discovery of SSV1 and it still represents a valid model to study the host–virus interaction in harsh environments (Martin et al. 1984; Ceballos et al. 2012; Schleper et al. 1992; Fröls et al. 2007a; Fusco et al. 2013). Moreover, so far it is the only member of the *Fuselloviridae* family showing a UV-inducible life cycle (Prangishvili 2013; Contursi et al. 2014a). Interestingly, the first proof of the existence of a UV-specific response in *Sulfolobus* was just derived from the transcription analysis of SSV1. In particular, the primary reaction after UV treatment of the host cells is the expression of a small transcript  $T_{\text{ind}}$ , which either acts as primer for viral replication and/or encodes for a UV-responsive transcription factor (Fröls et al. 2007a).

However, the UV irradiation exerts effect not only on the SSV1 induction but also on the host metabolism and vitality as well. Indeed, the transcriptional response in *Sulfolobus* cells is paralleled by a phase of marked growth retardation with DNA replication and cell division slowing down (Fröls et al. 2009). Exposure of cells to UV light causes the formation of two prevalent DNA lesions, i.e. cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts, the former of which has been detected in UV-irradiated cells of *S. solfataricus* (Salerno et al. 2003). The best characterized UV-damage repair system is the nucleotide excision repair (NER) pathway. Noteworthy, the genome of *S. solfataricus* encodes for homologues of the eukaryal NER system, which were found to be transcriptionally

up-regulated upon UV irradiation (Salerno et al. 2003). In *Eukarya* the contribution of the NER pathway to the removal of UV-induced DNA lesions is dependent on the magnitude of the UV exposure used, i.e. on irradiance and fluence (Lee et al. 2004). Similarly, it has been shown that these two parameters represent key factors in the activation of the c-Jun N-terminal kinase (JNK), which is mediated by DNA lesions in mammalian cells. In particular, a prolonged activation of JNK was revealed when the UV fluence was administered using a lower irradiance (Adler et al. 1996). Notably, studies on UV response in *Eukarya* have evidenced that conflicting data are produced when different experimental procedures are used (Lee et al. 2004). Similar discrepancies in *S. solfataricus* (Fröls et al. 2007b, 2009; Götz et al. 2007; Salerno et al. 2003) might be attributed to the lack of a standardized protocol. Indeed, in previous literature the fluence has been only empirically determined, whilst the irradiance has not been taken into account (Martin et al. 1984; Reiter et al. 1987; Schleper et al. 1992; Fröls et al. 2007a).

In this manuscript, we describe a suitable UV irradiation procedure, which is based on instrumentally measured parameters, i.e. the fluence and irradiance. A clear dose–response relationship between the UV irradiation and the host survival percentage is shown. Interestingly, cells lethality was significantly reduced through the tuning of the irradiance ( $0.5$  or  $1.0 \text{ J m}^{-2} \text{ s}^{-1}$ ) (Table 2). By analogy with eukaryal systems, this effect might be due to an improved functionality and/or activation of the DNA-damage repair systems (Lee et al. 2004; Adler et al. 1996).

A fluence of  $45 \text{ J m}^{-2}$ , in combination with an irradiance of  $0.5 \text{ J m}^{-2} \text{ s}^{-1}$ , turned out to be not only suitable to preserve host viability, but also to lead to the highest accumulation of viral DNA and of viral particles (Fig. 2; Table 3). Indeed, densitometric analysis of the PCR products showed an increase of the SSV1 copy number of about 32-fold, i.e. of  $\sim 160$  viral genome copies per cell in the irradiated culture collected 8 h post-irradiation. Moreover, the viral titre determined under the same irradiation conditions (about

**Table 3** Viral titre at 8th and 24th hours after UV treatment

Total fluence ( $\text{J m}^{-2}$ )	PFU/ml 8th Avg $\pm$ SD ( $n = 3$ )	PFU/ml 24th Avg $\pm$ SD ( $n = 3$ )	PFU/ml fold of increase 8th/24th
0	$2.10 \times 10^7 \pm 0.12 \times 10^7$	$2.45 \times 10^7 \pm 0.18 \times 10^7$	1.17
30 <sup>a</sup>	$2.03 \times 10^7 \pm 0.16 \times 10^7$	$8.26 \times 10^7 \pm 0.13 \times 10^7$	4.07
45 <sup>b</sup>	$2.31 \times 10^8 \pm 0.25 \times 10^8$	$4.91 \times 10^9 \pm 0.11 \times 10^9$	21.26
45 <sup>a</sup>	$3.85 \times 10^7 \pm 0.15 \times 10^7$	$6.35 \times 10^8 \pm 0.20 \times 10^8$	16.49
60 <sup>b</sup>	$3.92 \times 10^7 \pm 0.11 \times 10^7$	$5.03 \times 10^8 \pm 0.13 \times 10^7$	12.83
60 <sup>a</sup>	$1.76 \times 10^7 \pm 0.16 \times 10^7$	$3.68 \times 10^7 \pm 0.17 \times 10^7$	2.09
75 <sup>b</sup>	$1.81 \times 10^7 \pm 0.08 \times 10^7$	$2.94 \times 10^7 \pm 0.16 \times 10^7$	1.62

<sup>a</sup> Cells irradiated with an irradiance of  $1.0 \text{ J m}^{-2} \text{ s}^{-1}$

<sup>b</sup> Cells irradiated with an irradiance of  $0.5 \text{ J m}^{-2} \text{ s}^{-1}$

5  $10^9$  PFU/ml, Table 3), was one order of magnitude higher than previously reported for *S. solfataricus* (Schleper et al. 1992). Therefore, the enhanced viability of the cell population affects viral replication and virions extrusion, thus influencing the increase of both copy number and viral titre.

Altogether these data highlight the necessity of standardizing the irradiation procedure to better compare results from different research groups.

Moreover, the establishment of a standardized protocol for SSV1 induction might have biotechnological potentialities since it allows the isolation of a huge amount of viral DNA as well as of viral particles to be employed for genetic manipulation and nanoparticles production, respectively. In this regard, viral particles from hyperthermophilic *Archaea* have been demonstrated to be exploitable for the fabrication of new nanoparticles. In particular, the rod-shaped virus SIRV2 (*Sulfolobus islandicus* rod-shaped virus 2) has been referred as a novel nanobuilding block (Evans 2009). Similarly, the virion of SSV1 can be considered, by its nature, a stable nanoparticle that is resistant to low pH and high temperature, and is, therefore, a good candidate for future biotechnological applications.

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