Interplay between the genetic clades of *Micromonas* and their viruses in the Western English Channel

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Abstract

The genus Micromonas comprises distinct genetic clades that commonly dominate eukaryotic phytoplankton community from polar to tropical waters. This phytoplankter is also recurrently infected by abundant and genetically diverse prasinoviruses. Here we report on the interplay between prasinoviruses and Micromonas with regards to the genetic diversity of this host. During one year, we monitored the abundance of 3 clades of Micromonas and their viruses in the Western English Channel both in the environment, using clade-specific probes and flow cytometry, and in the laboratory, using clonal strains of Micromonas clades to assay for their viruses by plaque-forming units. We showed that the seasonal fluctuations of Micromonas clades were closely mirrored by the abundances of their corresponding viruses, indicating that the members of Micromonas genus are susceptible to viral infection, regardless of their genetic affiliation. The characterization of 45 viral isolates revealed that Micromonas clades are attacked by specific virus populations, which exhibit distinctive clade specificity, life strategies, and genetic diversity. However, some viruses can also cross-infect different host clades suggesting a mechanism of horizontal gene transfer within Micromonas genus. This study provides novel insights into the impact of viral infection for the ecology and evolution of the prominent phytoplankter Micromonas.

Key words: Mamiellophyceae, Phycodnaviridae, Prasinovirus, host specificities, life history traits, DNA polymerase, English Channel

Introduction

Viruses are undoubtedly the most abundant biological entities in the ocean and they intimately interact with every facet of the marine biosphere. Through these interactions, viruses profoundly influence the global biogeochemical cycles by altering the structure and the function of marine communities and by contributing to the cycling of major elements (for review see Suttle, 2005, 2007; Sime-Ngando, 2014). In spite of their global-scale implications, the nature and the dynamics of virus-host interactions in marine environments are far from understood and many basic, yet fundamental, questions remain unsolved: Who are the hosts infected by marine viruses? What are the infection strategies evolved by viruses? How do virus-host interactions vary in time and space? In this respect, characterizing the interplay of ecologically relevant virus-host model systems is a prerequisite for advancing our understanding of virus impacts in nature.

Viruses that infect the cosmopolitan green picoalga *Micromonas* were first reported by (Pienaar, 1976) and they were found to co-occur with their hosts in a wide range of marine environments, from temperate to cold waters (e.g., Cottrell and Suttle, 1991, 1995a; Sahlsten, 1998; Zingone *et al.*, 1999). The majority of known *Micromonas* viruses belong to the genus *Prasinovirus* in the Phycodnaviridae family. Virions are tailless, they exhibit icosahedral capsids of 100 -125 nm in diameter and dsDNA genome of approx. 200 kb (Mayer and Taylor 1979; Cottrell and Suttle 1991; Zingone *et al.*, 1999). Yet, these prasinoviruses have variable tolerance to chloroform (Martinez et al. 2015) suggesting ultrastructural divergence and they exhibit significant level of genetic variation (Cottrell and Suttle, 1995b). These viruses display a marked seasonal dynamics (Sahlsten, 1998; Zingone *et al.*, 1999) and they were shown to cause considerable amount of mortality in their host population (Cottrell and Suttle, 1995a; Evans *et al.*, 2003). *Micromonas* strains show a great variability with respect to their susceptibility to infection, indicating that viruses do not only quantitatively but also

qualitatively regulate their host populations (Sahlsten, 1998; Zingone *et al.*, 1999, 2006). So far, the infection patterns and processes underlying these complex interactions are poorly understood. Of particular interest, the relation between the susceptibility to infection and the genetic diversity of *Micromonas* has seldom been investigated (Zingone *et al.*, 2006; Martinez Martinez et al. 2015).

The ubiquitous genus *Micromonas* is genetically diverse and comprises an assemblage of 3 (Guillou *et al.*, 2004) to 5 (Šlapeta *et al.*, 2006; Worden, 2006) discrete phylogenetic clades (or lineages) of flagellated cells that correspond, at least, to 3 distinct species (Simon, unpublished). These clades are often sympatric in marine ecosystems (Foulon *et al.*, 2008; Šlapeta *et al.*, 2006). Yet, their relative contribution to total *Micromonas* abundance varies in time and space, suggesting that they occupy specific niches (Foulon *et al.*, 2008). The factors that regulate this clade dynamics are not clearly understood. In all likelihood, *Micromonas* clades exhibit differential responses to abiotic factors but they might also respond differently to predation risks, including those imposed by viruses.

To test whether *Micromonas* clades display differential susceptibility to virus infection, we combined field and laboratory experiments on 45 novel viral isolates in order to characterize the interactions between the genetic clades of *Micromonas* and their viruses in the Western English Channel (WEC) throughout the year 2009. The WEC constitutes an ideal study site where *Micromonas* is known to dominate the picophytoplankton community (Not *et al.*, 2004). The three main *Micromonas* genetic types, designated as clades [A.ABC.12], [B.E.3], and [C.D.5] (Worden, 2006) and here referred to as clades A, B, and C, respectively, are recorded year round in this ecosystem (Foulon *et al.*, 2008). Our study revealed that *Micromonas* clades interact with specific viral populations that display distinctive dynamics and life history traits.

Results and Discussion

Physico-chemistry at the sampling station

Strong tidal mixing produces a permanently mixed water column at the long term monitoring coastal station SOMLIT-Astan (48°46′N, 3°58′W, Marrec *et al.*, 2013). During the sampling period (February to December 2009), the water temperature progressively increased from 8.8°C in February to a maximum of 16.9°C in September while salinity varied between 34.8 (March) and 35.3 (September). Nutrient dynamics varied according to the classical pattern observed in this area (Not *et al.*, 2004). Phosphate and nitrate concentrations showed comparable dynamics with minima recorded in summer (0.16 and 0.7 µM, respectively) and maxima during the winter period (0.53 and 11 µM, respectively). Detailed dynamics of biogeochemical variables at SOMLIT-Astan are publicly available on the observatory website (http://somlit-db.epoc.u-bordeaux1.fr/bdd.php).

Dynamics of *Micromonas* clades in the Western English Channel

The phytoplankton community at SOMLIT-Astan during this period was numerically dominated by picophytoplanktonic cells, which comprised, on average, 90% of the total community in 2009 (data not shown). Among these, the abundance of the picoeukaryotes varied between 2.1 and 16.5 × 10^3 cells mL^{-1} with *Micromonas* spp. accounting for 20% to 80% of the total counts as shown by a combination of flow cytometry (FCM) and TSA-FISH analyses (Fig. 1A). As reported previously, this prominent genus displayed marked seasonal dynamics with major peaks of abundance recorded in late spring (6.6 × 10^3 cells mL^{-1}), early summer (6.3 × 10^3 cells mL^{-1}), and mid-autumn (4.4 × 10^3 cells mL^{-1}) (Foulon *et al.*, 2008; Not *et al.*, 2004).

The 3 main *Micromonas* genetic clades (hereafter referred to as clades A, B, and C) exhibit recurring seasonal dynamics in the WEC according to previous time series (Foulon *et al.*, 2008). We confirmed this pattern of succession in 2009 (Fig. 1B). *Micromonas* clade A numerically dominated the bloom from February to mid-May comprising 63 to 77% of the total counts. From June to December, both genetic types A and B equally contributed to *Micromonas* counts, although clade B cells showed a sudden, yet unexplained, drop in abundance on June 16. Cells that belonged to clade C were the least abundant accounting, on average, for 7 ± 6% of the total counts with somewhat higher contribution during the winter period (14%) than the remainder of the year (5%).

The ecological processes that influence the shifts in the relative abundance of *Micromonas* clades are unclear. It is traditionally believed that global-scale distribution and diversity of species are mostly driven by ability to withstand abiotic controls (Wiens et al. 2011). Previous study on the global distribution of *Micromonas* clades suggested that clade B tend to thrive in warmer coastal waters whereas clade C mostly occur during low-light conditions (Foulon *et al.*, 2008). At local spatial extents, it is however well accepted that species distribution is also influenced by biotic controls (Wiens et al. 2011). It is thus likely that besides eco-physiological adaptations, biotic interactions also regulate the observed *Micromonas* clade dynamics in the Western English Channel.

Dynamics of *Micromonas* viruses in the Western English Channel

Viruses that infect *Micromonas* (hereafter referred to as MicV), mainly prasinoviruses, have been observed and isolated from different oceanic regions (Cottrell and Suttle, 1991, 1995a; Sahlsten, 1998; Zingone *et al.*, 1999; Short and Short, 2008). FCM analyses of cultured isolates showed that these prasinoviruses consistently cluster within a well-defined population based on their nucleic acid fluorescence (upon Sybr green I staining) and side scatter properties (Fig. S1). In the WEC, this specific population accounted for a small but

significant proportion (on average 10%) of the total virus counts and displayed a marked seasonal dynamics with concentration ranging from 3.4×10^5 mL^{-1} in February to 8.8×10^5 mL^{-1} in July 2009 (Fig. 2A). This FCM population probably does not exclusively comprise *Micromonas* viruses, but counts of this viral population covary strikingly well with *Micromonas* abundances ($r=0.675$, $n=20$, $p<0.005$), suggesting that viruses might perhaps control abundance of this genus during the sampling period.

To further investigate this hypothesis, we quantified infectious MicV in the WEC by plaque assay using cultures of each 3 *Micromonas* clades. Significant differences in the number of PFU were detected depending on the phylotype of the host culture (Fig. 2B). As also recorded for their hosts, PFU obtained on *Micromonas* clade A and B (hereafter, PFU-A and PFU-B, respectively) were substantially more abundant than PFU formed on clade C-hosts (hereafter PFU-C). During the sampling period, PFU-A abundance increased concomitantly with the development of *Micromonas* clade A. Their maximum abundance (8×10^2 virus mL^{-1}) was observed 2 weeks after the peak of host abundance (Figs. 1B and 2B). The monitoring of PFU-B showed low concentration during the winter-spring period but their dynamics was tightly coupled to their host abundance from the summer period until the end of the year, reaching a peak of 11×10^2 virus mL^{-1} in autumn. Regarding PFU-C, infectious particles were detected year round but their abundance was 10 to 100-fold lower than PFU-A and -B with no clear relation to their specific host dynamics. Because of variable strain susceptibilities to viral infection, it is difficult to compare our results to former PFU monitoring on *Micromonas* host cell clades although maximal abundance were within the same range ($1.0 - 4.6 \times 10^3$ PFU mL^{-1}, Cottrell and Suttle, 1991; Sahlsten, 1998; Zingone *et al.*, 1999). For the same reason, this assay most likely underestimates the global abundance of viruses that infect each *Micromonas* phylotype. Nonetheless, it provides unequivocal evidence

that *Micromonas*, regardless of their genetic clade, were subject to viral attack year round in the WEC.

Micromonas virus - host interactions

The above results led us to question whether specific virus populations are associated to *Micromonas* clades and, if so, how these virus populations interact with their hosts. To tentatively address this question, 45 viruses (14-16 per host clade) were isolated throughout the sampling period and they were characterized. These viruses were selected randomly from a collection of 176 viral isolates obtained from plaques on *Micromonas* clade A, B, and C along the year 2009. Regardless of the host on which they were isolated, MicV isolates exhibited sizeable icosahedral capsids (110 to 130 nm in diameter) as determined by negative staining using transmission electron microscopy, and large dsDNA genome, as suggested by their flow cytometry signature upon Sybr green I staining (Fig. S1). We therefore assigned our isolates to the virus family Phycodnaviridae and the genus *Prasinovirus*.

Host specificities - The host range of these 45 prasinoviruses was then determined by pair-wise infection using 14 strains belonging to the 3 *Micromonas* clades (Table 1, Table S1). The large majority of our isolates (35 MicVs) were strictly clade-specific. They infected host strains that belonged only to the clade of their initial host. The remaining 10 viruses could propagate at comparable yield on hosts belonging to clades A and B. Six viruses (out of 15) isolated on a clade A strain could also infect clade B strains, and 4 viruses (out of 14) isolated on a clade B strain could also infect clade A strains. Within each main pattern of specificity (A, B, AB, and C), a remarkable clonal diversity was observed with 26 unique specificity profiles that included variable virus infection types ranging from generalist to specialist.

In order to test the structure of this infection network, we applied a network-based analysis according to Flores *et al.* (2011) and Weitz *et al.* (2013). As observed previously for phage-bacteria infection networks (Flores *et al.*, 2011), the structure of *Micromonas* - virus interactions was statistically modular. This statistical analysis applied at the whole matrix scale and intra-module scale discriminated 3 modules, which comprised the viruses isolated on hosts from clade A, clade B, and clade C, respectively (Fig. S2). Altogether, these results suggest that *Micromonas* clade A, B, and C interact with distinct, yet diversified, viral populations. The observed variability in strain-specificity indicates that viruses influence the intraspecific diversity within each *Micromonas* clades. We also showed that host switches can occur between the more closely related host clades (those that belong to clades A and B), suggesting that viruses could also promote *Micromonas* diversity through horizontal gene transfers within this genus.

Virus infection strategies - One-step growth experiments conducted on 4 to 5 representative viruses per host clade indicated a similar clade-specific grouping of our isolates. This assay provided estimates of the virus burst size and latent period, which can be related to the virus life strategy. By analogy to the theory of the r- and K-selection (MacArthur, 1967), opportunistic (the most r-selected) viruses are those that exhibit short generation times and high burst sizes whereas less virulent (the most K-selected) viruses are those that induce low mortality in their host population (Suttle, 2007) and they propagate through latent or chronic infection as observed for specific *Ostreococcus* viruses (Thomas *et al.*, 2011). Based on these parameters, we detected divergent life strategies in *Micromonas* viruses depending on the host clades that they attack (Fig. 3). The viruses isolated on clade B hosts tended to be the most virulent with short latent period and relatively high progeny production, regardless of their infection range types (i.e. specialist or generalist) whereas the viruses isolated on clade C

strains tended to be the least virulent with generally extended latent period (up to 30 h) and moderate to high burst size (Fig. 3). Viruses isolated on clade A hosts exhibited an intermediate phenotype with moderate latent period and low burst sizes.

In contrast to past theories (trade-offs hypothesis, (Poulin, 1998)), generalism (i.e., viruses that propagate on several host clades) was not associated with any compensatory effect on the burst size or on the latent period. The observed differences in viral infection kinetics did not correlate to host growth rates (0.6 -0.9 d^{-1}, Fig. S3). However, viral infection strategies appeared to be related to the dynamics of their respective host clade in the studied area. For example, *Micromonas* clade C is present year round at low abundance. It is thus tempting to speculate that viruses specific to this host clade had to evolve long latent periods to be maintained within the host population and fairly high progeny production to increase the probability of encounter with their host (Abedon *et al.*, 2001). By contrast, *Micromonas* clade B displays a marked seasonal dynamics in the WEC with high abundance recorded between mid-June and end-September. We could thus expect that corresponding viruses had to evolve an opportunist lifestyle to efficiently exploit their transient resources.

Phylogenetic relationships between *Micromonas* viruses

To investigate the genetic diversity among our viral isolates and to determine how this diversity relates to the virus biological properties, we amplified a fragment of the gene encoding their DNA polymerase (*polB*, Chen and Suttle, 1996, Table S2). This sequence is well conserved among Phycodnaviridae, yet there is sufficient sequence variability to build phylogenetic relationships between viruses from the same genus (Bellec *et al.*, 2009, Clerissi *et al.*, 2014). In spite of their affiliation to the Phycodnaviridae family, the amplification of the *polB* gene using the AVS1/AVS2 primers failed for 8 of the 45 selected isolates including B-, C- and AB-specific viruses. The reason for this failure is unclear. We cannot exclude that

the sequence targeted by the degenerated primers might be too divergent. It is also possible that these polymerases carry an intein that makes the PCR product too long to amplify easily under the tested conditions (Clerissi *et al.*, 2013).

The partial *polB* sequences of the remaining 37 isolates fell into 28 haplotypes (Fig. 4). In most cases, viral isolates that belonged to the same haplotype exhibited different specificity patterns, suggesting that *polB* sequencing underestimates the functional diversity of phycodnaviruses as reported previously (Baudoux and Brussaard, 2005; Bellec *et al.*, 2009; Clerissi *et al.*, 2012). In the phylogenetic trees, *polB* sequences of viruses infecting clade C hosts formed a well-supported cluster while viruses that infect clade A and clade B hosts distributed into multiple branches suggesting a higher level of genetic diversity. Interestingly, regardless of the host strain they infect, viruses that were highly specific (that infect one single strain among all strains tested) fell apart from other viruses (i.e. RCC4232 and RCC4240, 4242, 4243, Fig. 4 and Table 1). The prasinoviruses that infect *Ostreococcus tauri* showed a similar phylogenetic pattern and tended to cluster with counterparts that exhibit similar infection range types (Clerissi *et al.*, 2012).

This recurrent correlation between *polB* phylogeny and the number of hosts infected by a given prasinovirus is somewhat puzzling. The *polB* gene is indeed used as a neutral marker of evolution in Phycodnaviridae (Chen and Suttle, 1996; Dunigan *et al.*, 2006), that is, this gene is well-conserved among this virus family whereas specificity markers are expected to be more variable genes (Clerissi *et al.*, 2012). Furthermore, virus-host specificity is typically determined during events upstream the virus DNA replication. Several studies reported that the virus attachment on host cell surface determines the susceptibility of marine plankton to infection (e.g., Tarutani *et al.*, 2006; Mizumoto *et al.*, 2007; Stoddard *et al.*, 2007). In other words, the evolution of resistance to viral infection is mostly due to changes in host surface properties that limit viral attachment. Recently, Thomas *et al.* (2011) evidenced

an intriguing mode of resistance in the prasinophyte *Ostreococcus tauri*. This study demonstrated that prasinoviruses could still attach on resistant strains but they did not produce viral progeny. It is thus likely that the resistance mechanism was not due to a change in the host receptor but rather occurred at the DNA entry or DNA replication stage, in which the *polB* gene product would be involved. Alternatively, viral attachment and internalisation might be a two-stage process as in reoviruses (Reiter *et al.*, 2011). The notion that DNA replication machinery might be involved in host specificity is of course highly speculative but the repeatedly observed link between *polB* clustering and prasinovirus infectivity range certainly deserves appropriate investigation.

Concluding remarks

In the Western English Channel, a complex assemblage of viruses is associated to the dominating picophytoplankton species *Micromonas spp*. Our study revealed, for the first time, that the main *Micromonas* genetic clades (clade A, clade B, and clade C) are attacked by specific virus populations which displayed distinctive dynamics and life history traits. The apparently high variability in *Micromonas* virus specificities suggests that viruses maintain a high genetic diversity within each of these clades. However, host switching can occur (particularly for hosts that belong to the clade A and B) suggesting that virus could also influence *Micromonas* diversity through horizontal gene transfers. The complete genome sequencing of representative viruses and hosts should provide insightful information on the extent of these putative genetic exchanges. It is of course critical to keep in mind that our model-based approach only reflects a partial picture of the actual diversity of virus-host interactions. Still, these approaches provide invaluable information to improve our understanding of the mechanisms of virus-host interactions as well as the evolution and outcomes of these interactions in natural environments.

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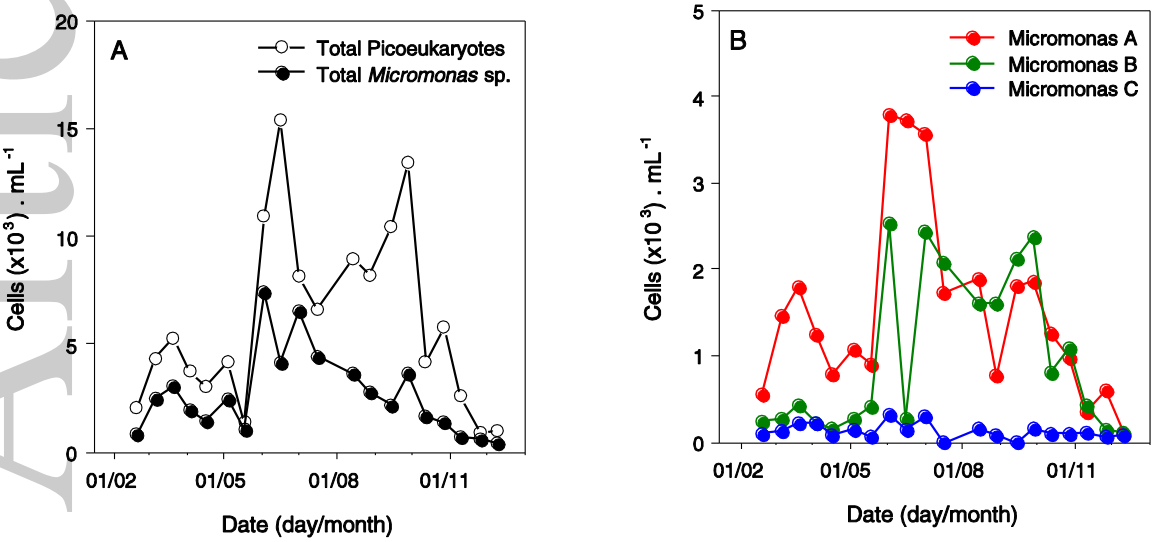

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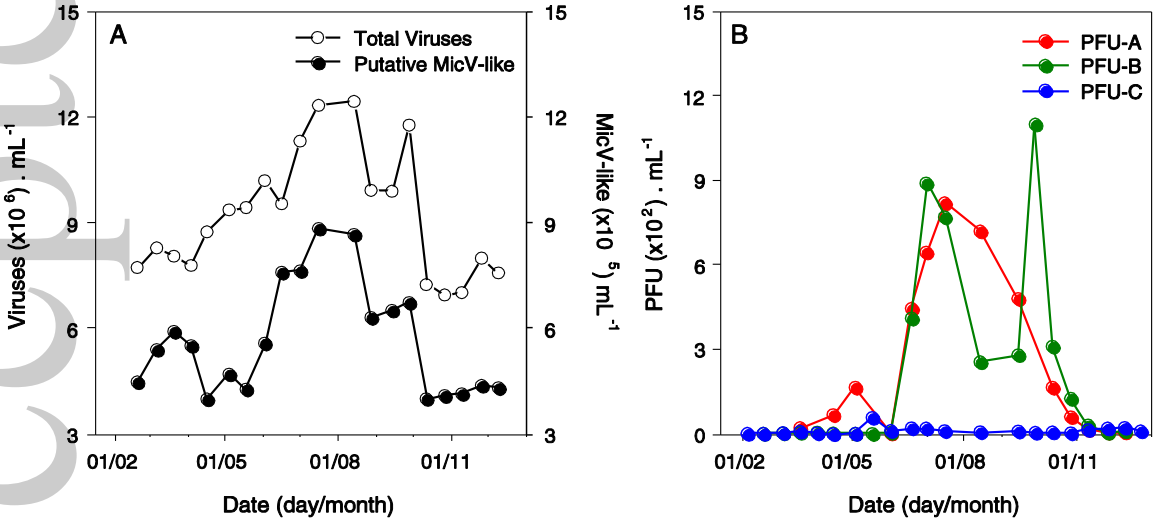

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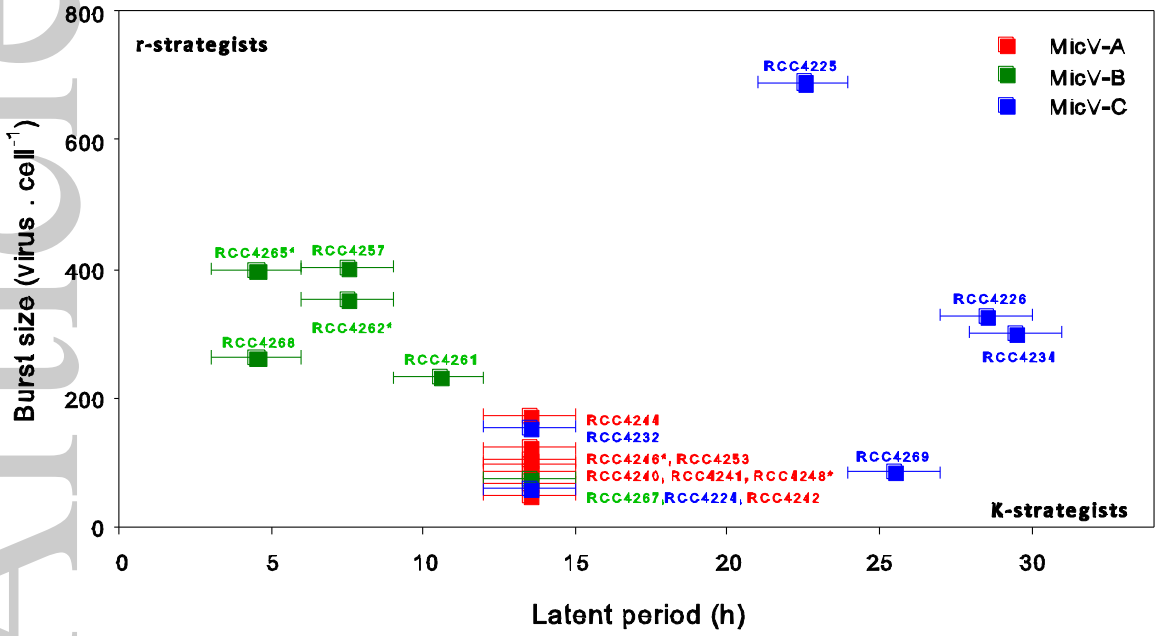

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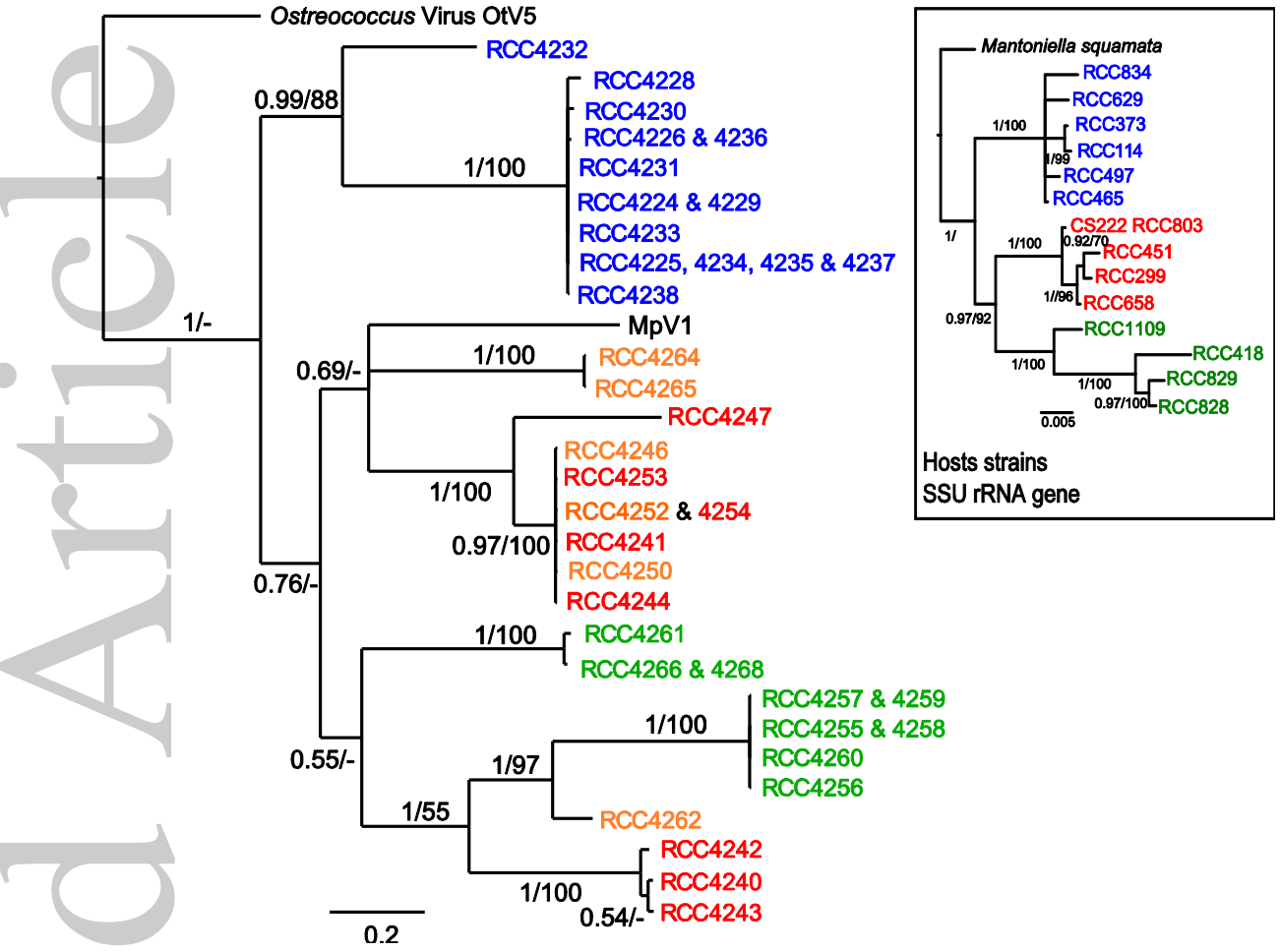

EMI4_12309_F4

Table 1: Host range of 45 *Micromonas* viruses isolated from SOMLIT-Astan throughout the year 2009. *Micromonas* viruses are ordered chronologically according to the host clade on which they were isolated. *Micromonas* strains are listed according to their genetic types based on 18S rRNA and ITS sequences (Table S1). Viruses were isolated from plaque assays and they were made clonal by repeated end-point dilution. For the pair-wise infection, freshly produced MicV isolate was added to exponentially growing algal cultures (MOI 10). Cultures that underwent lysis were inspected for viral proliferation using flow cytometry (Brussaard, 2004). ■ : infection; o : no infection, - : not determined