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Effects of pH and temperature on the survival of coliphages MS2 and Qβ

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Abstract The RNA F-specific coliphages, MS2 and Qβ, have been used as virus indicators in water and wastewater studies. It is therefore useful to have a good understanding concerning the effects of environmental factors on their survival in order to choose an appropriate candidate for assessing microbial safety in relation to water quality management. The effects of pH and temperature on the survival of these two coliphages were investigated. MS2 survived better in acidic conditions than in an alkaline environment. In contrast, Qβ had a better survival rate in alkaline conditions than in an acidic environment. The inactivation rates of both coliphages were lowest within the pH range 6–8 and the temperature range 5–35°C. The inactivation rates of both coliphages increased when the pH was decreased to below 6 or increased to above 8. The inactivation rates of both coliphages increased with increasing temperature. Qβ behaved peculiarly in extreme pH buffers, i.e. it was inactivated very rapidly initially when subjected to an extreme pH environment, although the inactivation rate subsequently decreased. In general, MS2 was a better indicator than Qβ. However, within the pH range 6–9 and at temperatures not above 25°C, either MS2 or Qβ could be used as a viral indicator.

Keywords Coliphage MS2 · Coliphage Qβ · pH Temperature · Inactivation

Introduction

Enteric viruses have been responsible for many waterborne disease outbreaks. However, it is often not practical to detect viruses directly because the tests are time-consuming, expensive, difficult to perform, and dangerous to operators due to their infectivity [20]. In view of this, it is desirable to use appropriate virus indicators for assessing the performance of treatment systems in terms of microbial safety. F-Specific bacteriophages (e.g. MS2, R17 and Qβ) have been recommended for modeling viral behavior in water because their size and structural properties are similar to many of the human enteric viruses, and they can be quantified more easily and rapidly [11,22].

MS2 has been widely used as an indicator in various investigations, such as virus transport through soil [17], membrane filtration [18], and in a disinfection study [9]. It has also been used as an indicator of enteric viruses in various wastewater treatment systems such as primary and secondary treatment, lime treatment, oxidation ponds and wetlands [7,14,15]. It could also act as a virus indicator for fresh water, seawater and underground water studies due to its long survival time in these waters [12]. Compared with MS2, another potential virus indicator, coliphage Qβ, has seldom been studied although its application in membrane separation processes [18] and its replication properties [21] have been reported. Similar to MS2, it also displays morphological and structural resemblances to enteric viruses. It belongs to the RNA F-specific coliphages, and is likely to be highly resistant to unfavorable environmental conditions [5]. Hence, it too shows good potential as a virus indicator.

Although MS2 is a common and widely used indicator in water-related studies, and Qβ is a potential indicator for similar studies, the influences of environmental factors on their survival have not been systematically studied. The objective of this study was therefore to investigate the effects of pH and temperature on MS2 and Qβ survival, and to compare the two coliphages to determine which is the better virus indicator based on their inactivation rates under specific environmental conditions. This information will be useful for water treatment, storage, and other water related research.

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Materials and methods

Preparation and assay of coliphage MS2 and Qβ

MS2 (ATCC 15597-B1) was propagated for use by inoculating 1 ml host bacteria (*Escherichia coli* ATCC 15597) into a 500 ml flask containing 100 ml tryptic soy broth (TSB). The culture flask was placed in a shaking incubator maintained at 37°C. When the bacterial density reached approximately 1×10^8 cfu/ml, an aliquot of virus stock (approximately 10^{11} pfu/ml) was added to provide a multiplicity of infection of 10. The culture was shaken continuously until the host cells lysed. The propagated virus and cellular debris were then centrifuged for 20 min at 2,608 *g* and filtered through a sterile 0.22 μm pore size filter. The resultant stock was titered via the agar overlay technique and refrigerated at 4°C until needed [1]. Qβ (ATCC 23631-B1) was propagated and assayed in the same way by using *E. coli* (ATCC 23631) as the host.

Experimental design

For studies on the effect of pH, two types of buffer solutions were prepared, i.e. Clark and Lubs buffer solutions (pH 3–8) and alkaline buffer solutions (pH 9–11). The buffers were used at a final concentration of 50 mM: potassium hydrogen (KH) phthalate/HCl for pH 3–4, KH phthalate/NaOH for pH 5, KH_2PO_4/NaOH for pH 6–8, Tris(hydroxymethyl)-aminomethane/HCl for pH 9, and $NaHCO_3$/NaOH for pH 10–11. Buffer (500 ml) was stored in a 1 l Pyrex glass beaker. As the buffer solutions did not contain NaCl, they are unlikely to affect the inactivation process [19]. For temperature effect studies, the buffers were kept at 5°C, 15°C, 25°C, and 35°C. A 1 ml aliquot of MS2 or Qβ stock solution was added to the various samples at time zero. At time intervals, 2-ml samples were assayed to determine concentrations of MS2 or Qβ. The experiments were conducted in triplicate.

Data analysis

Linear regression analyses were used to calculate inactivation rates for each experimental study. The inactivation rate (k) was expressed by the equation: $k = -(\log_{10} N_t / N_0)/t$, where N_t and N_0 are the final and initial numbers of viruses per milliliter volume of water respectively, and t represents time in days. For non-linear relationships (tailing-off curve), the maximum inactivation rate was calculated by the same equation based on the initial linear phase.

Results

The inactivation rates obtained are presented in Fig. 1. The relationship between the log of MS2 concentration and time was approximately linear over the pH (3–11) and temperature (5–35°C) values studied, while this was not always the case for Qβ. Although a linear relationship between the log of Qβ concentration and time existed for most of the pH and temperature values studied (pH range 5–10 in the temperature range of 5–35°C, and for pH 11 under the temperature range of 5–15°C), a linear relationship did not hold for pH 3–4 under the temperature range 5–35°C and for pH 11 under the temperature range 25–35°C. In non-linear situations, Qβ was inactivated at a higher rate initially, but this high inactivation rate decreased sharply after some time although Qβ could still be completely inactivated

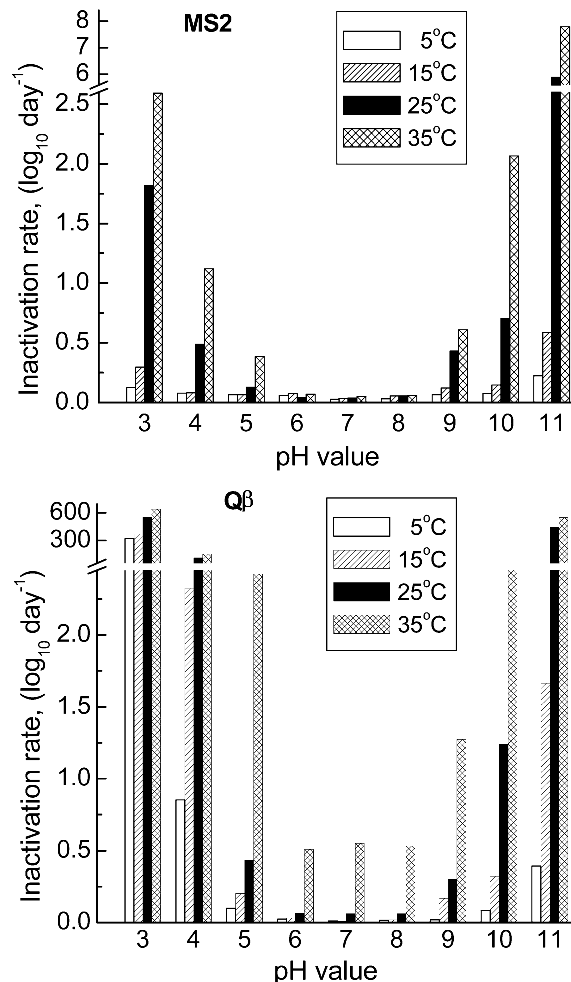

Fig. 1 Inactivation rate constants of coliphages MS2 (*top*) and Qβ (*bottom*)

within a relatively short period. In order to compare the inactivation rates in non-linear situations with those in linear situations, the inactivation rates in non-linear situation were calculated based on the data collected in the initial linear range (from time zero to a time when the linear relationship vanished). At pH 3 under the temperature range of 5–35°C, and pH 11 under the temperature range of 25–35°C, the turning point (the point at which the linear relationship vanished) occurred 20 mins after the start of the experiment. The turning points for pH 4 were 2 days and 60 mins for temperature ranges of 5–15°C and 25–35°C, respectively.

The inactivation rates of both MS2 and Qβ were lowest within the pH range 6–8 at all temperatures studied (Fig. 1). However, inactivation rates increased when the pH deviated from near neutral conditions. Outside the near neutral environment, MS2 survived better at an acidic pH than an alkaline pH, in the temperature range 5–35°C. For example, at 25°C the inactivation rate of MS2 at pH 9 was 3.3 times greater than that observed at pH 5. In contrast, Qβ was the opposite as it survived better in an alkaline pH than in an acidic pH over the same temperature range. For example, at 25°C the inactivation

rate of Qβ at pH 5 was 1.4 times greater than that observed at pH 9. It was noted that, at the same temperature and pH, Qβ tended to die off faster than MS2. More specifically, in acidic buffers, Qβ died off much faster than MS2. Although the difference was less significant compared with the case of acidic pH, Qβ still died off faster than MS2 in the near neutral pH environment. Depending on temperature, the Qβ inactivation rate could be slightly higher or lower than that of MS2 when it was present in slightly alkaline buffers (e.g. pH 9). At high pH (10 or 11), Qβ died off at a much higher rate than MS2. It can also be seen from Fig. 1 that the inactivation rates of both MS2 and Qβ tended to increase with increasing temperature over the range of pH tested.

Discussion

Substances or conditions that denature proteins or react chemically with proteins or nucleic acids will inactivate phages. The inactivation of MS2 and Qβ observed in this study could be attributed to reactive radicals and levels of heat stability. Temperature has a major effect on the effectiveness and/or the rate of kill of a given microorganism because it controls the rate of chemical reactions. Thus, as temperature increases, the rate of kill induced by a chemical will also increase. In addition, pH can affect the ionisation of chemicals. At extreme pH values, the high concentrations of hydrogen ion and hydroxyl ion present in water are considered to be far greater than the concentration of free reactive radicals and therefore dominate viral inactivation mechanisms. Previous work [8] has shown that highly reactive radicals in a water environment (including hydroxyl and superoxyl ions) have relatively long lifetimes. These reactive radicals can oxidise materials in the water environment. Therefore, at neutral and slightly acidic or alkaline pH values, the virus protein coat may have been affected by removal, deformation or denaturation of some critical sites. After the protein coat is compromised, RNA hydrolysis may occur inside or outside the virus particle, which in turn results in loss of infectivity [19]. In the case of extreme pH values, the virus surfaces (capsid, tail fibres, etc.) may be attacked through the mechanism of direct oxidation when exposed to these environments, and dissociation of the capsid would therefore occur [3,13,16].

MS2 and Qβ are recommended for modeling viral behaviour in water because of their similar size and structural properties to many of the human enteric viruses. The sizes of MS2, Qβ, and enteroviruses are 26.0–26.6 nm, 26 nm, and 20–30 nm, respectively. They are all icosahedral in morphology, with no envelope, and with a single-stranded RNA genome. They all infect the host cell through an F pilus [5]. However, our results indicate that the pH and thermal stability of MS2 and Qβ are quite different. MS2 was generally more stable than Qβ over the pH and temperature ranges studied. The differences in pH and thermal stability of MS2 and Qβ were possibly due to (1) the different molecular weights of the capsid protein (13,731 Da for MS2 and 14,125 Da for Qβ) and the A protein that acts as an attachment organ for tail-less phages (43,988 Da for MS2 and 41,000 Da for Qβ), and (2) differences in protein components, as reflected by different isoelectric points (pI) (3.9 for MS2 and 5.3 for Qβ) [5]. These slight differences could have an effect on their behaviour under conditions of environmental stress. In addition, MS2 was more stable at acidic pH than at alkaline pH, while Qβ was just the opposite. Therefore, compared with Qβ, MS2 could better simulate enteroviruses in their pH stability because enteroviruses are recognised as acid-stable [19].

A non-linear relationship between the log of Qβ concentration and time was observed at pH 3 and pH 4 from 5°C to 35°C, and at pH 11 from 25°C to 35°C. Under these conditions, the initial inactivation rate of Qβ was very rapid followed by a much slower rate. This phenomenon could be attributed to viral aggregation [10]. Perhaps these viral aggregates provided shelter to some organisms from the unfavourable conditions. The aggregation by Qβ could be viewed as a natural defence mechanism for protection from unfavourable conditions. This observation was consistent with microbial responses to other forms of stress, many of which also result in aggregation or particle association [2,4,6].

The purpose of this study was to understand the survival rates of MS2 and Qβ at different pH and temperature values so that we could find a better indicator of water quality. Our results obtained indicate that there is no single answer regarding which coliphage is the better indicator. The characteristics of the water and wastewater as well as the environmental conditions should be taken into account in order to choose an appropriate indicator virus. If the pH of the water is within the range 6–9, and the temperature is not above 25°C, either coliphage can be used. In contrast, in extreme acidic or alkaline pH conditions, or when temperature is higher than 25°C, MS2 would be the better indicator. Indeed, if the presence of enteroviruses is the only concern, then MS2 is generally a better choice. The results obtained from this study should be useful to water research involving extreme pH conditions or large pH changes such as (1) lime treatment or lime softening where the pH is normally within the range 9–11, (2) some storage studies where the pH may gradually change from neutral to alkaline, or (3) monitoring of pathogen survival in reclaimed water where the pH could be slightly acidic.

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