

# His1 and His2 are distantly related, spindle-shaped haloviruses belonging to the novel virus group, *Salterprovirus*

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## Abstract

Spindle-shaped viruses are a dominant morphotype in hypersaline waters but their molecular characteristics and their relationship to other archaeal viruses have not been determined. Here, we describe the isolation, characteristics and genome sequence of His2, a spindle-shaped halovirus, and compare it to the previously reported halovirus His1. Their particle dimensions, host-ranges and buoyant densities were found to be similar but they differed in their stabilities to raised temperature, low salinity and chloroform. The genomes of both viruses were linear dsDNA, of similar size (His1, 14,464 bp; His2, 16,067 bp) and mol% G + C (~40%), with long, inverted terminal repeat sequences. The genomic termini of both viruses are likely to possess bound proteins. They shared little nucleotide similarity and, except for their putative DNA polymerase ORFs, no significant similarity at the predicted protein level. A few of the 35 predicted ORFs of both viruses showed significant matches to sequences in GenBank, and these were always to proteins of haloarchaea. Their DNA polymerases showed 42% aa identity, and belonged to the type B group of replicases that use protein-priming. Purified His2 particles were composed of four main proteins (62, 36, 28 and 21 kDa) and the gene for the major capsid protein was identified. Hypothetical proteins similar to His2 VP1 are present in four haloarchaeal genomes but are not part of complete prophages. This, and other evidence, suggests a high frequency of recombination between haloviruses and their hosts. His1 and His2 are unlike fuselloviruses and have been placed in a new virus group, *Salterprovirus*.

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## Introduction

At the extreme salinities found in salt lakes, the majority of cells are prokaryotes belonging to the archaeal family Halobacteriaceae (Grant et al., 2001). As in marine waters, the concentration of virus-like particles (VLPs) in salt lakes greatly exceeds that of the cells (Wommack and Colwell, 2000) and viruses appear to significantly limit the microbial population (Guixa-Boixareu et al., 1996). Direct electron microscopy of hypersaline waters has shown that spindle-shaped and round VLPs are the predominant morphotypes. Head-tail particles are less common (Guixa-Boixareu et al., 1996; Oren et al., 1997) but represent the majority of reported

halovirus isolates (Dyal-Smith et al., 2003; Witte et al., 1997; Zillig et al., 1988). The first spindle-shaped halovirus, His1, was reported in 1998 (Bath and Dyal-Smith, 1998) and the first round halovirus, SH1, was described in 2005 (Porter et al., 2005). However, several years before the His1 report, spindle-shaped viruses of *Sulfolobus* (a thermophilic crenarchaeon) had been discovered and subsequently studied in elegant detail (Palm et al., 1991; Stedman et al., 2003; Wiedenheft et al., 2004; Zillig et al., 1996). These crenarchaeal viruses were classified in a novel family of archaeal viruses, the Fuselloviridae, with the type species being SSV1 (for *Sulfolobus* spindle-shaped virus 1).

Since the isolation of SSV1, other spindle-shaped archaeal viruses (and VLPs) with similar properties have been described. These include several viruses of *Sulfolobus* that are similar to SSV1 (Prangishvili and Garrett, 2005; Stedman et al., 2003; Wiedenheft et al., 2004); PAV1, a *Pyrococcus* VLP (Geslin et al., 2003) that resembles SSV1 in genome

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size and structure (18 kb, circular, dsDNA); and a VLP observed in cultures of *Methanococcus voltae* A3 (Wood et al., 1989) that has a circular, dsDNA genome (23 kb) and can integrate into the host chromosome (as does SSV1). All of these examples can replicate without obvious lysis of host cultures.

The resemblance of His1 to SSV1 in morphology and genome size led us to propose that His1 be included in the Fuselloviridae family (Bath and Dyall-Smith, 1998) but it was not known at that time if His1 shared a similar replication strategy or any sequence identity with members of this family. Here, we present the isolation and characteristics of a second spindle-shaped halovirus, His2, and show that His1 and His2 are distantly related to each other but are not related to members of the Fuselloviridae or to other spindle-shaped viruses. We also show that their genomes are linear and, probably possess terminal-bound proteins, and are likely to replicate via a protein-primed DNA polymerase. In addition, the major capsid protein of His2 has homologues in three of the five completed haloarchaeal genome sequences.

## Results

### Isolation, host range and plaque morphology of His2

The Pink Lakes (36° 24.221' S, 141° 57.556' E) are a group of relatively small (1.3 km<sup>2</sup> or less), closely spaced salt lakes in a protected national park in the remote, semi-arid northwest of Victoria, Australia. In 1995, a water sample collected from these lakes was plated on overlay lawns of *Haloarcula hispanica*, *Haloferax volcanii* and *Haloferax lucentense*. After incubation (2 days, 37 °C), a single plaque formed on the *Har. hispanica* lawn, and from this a halovirus, designated His2, was isolated that produced clear plaques of about 2 mm diameter, with an undefined hazy edge. The plaques were quite distinct from those of His1 (Bath and Dyall-Smith, 1998), a halovirus of *Har. hispanica* that was isolated in southern Victoria (Fig. 1). The optimum plaquing temperature for His1 was 30 °C, while His2 plaqued best at 37 °C. The plaque morphology of His2 was the same at both temperatures, but plaques took longer to appear when plates were incubated at 30 °C.

The host range of His2 was investigated by spotting a high titred virus lysate (10<sup>10</sup> plaque-forming units (PFU) per ml) onto plate overlays containing one of 17 members of the family Halobacteriaceae (see Experimental procedures), including members of six genera and three additional species of *Haloarcula* (*Har. marismortui*, *Har. sinaiensis* and *Har. vallismortis*). Plaques were only observed on lawns of *Har. hispanica*.

His2-infected liquid cultures of *Har. hispanica* produced high virus titres (≥10<sup>10</sup> PFU/ml). The culture optical density increased over the first 24 h incubation then decreased to reach a minimum at 2–3 days. Cultures generally remained turbid but, in about 10% of cases, complete lysis would occur in small volume cultures (10 ml). Lysis did not produce higher virus titres. The clear plaques and occasional lysis of liquid cultures indicated that His2 was lytic in nature. Attempts to isolate lysogens from infected cultures were

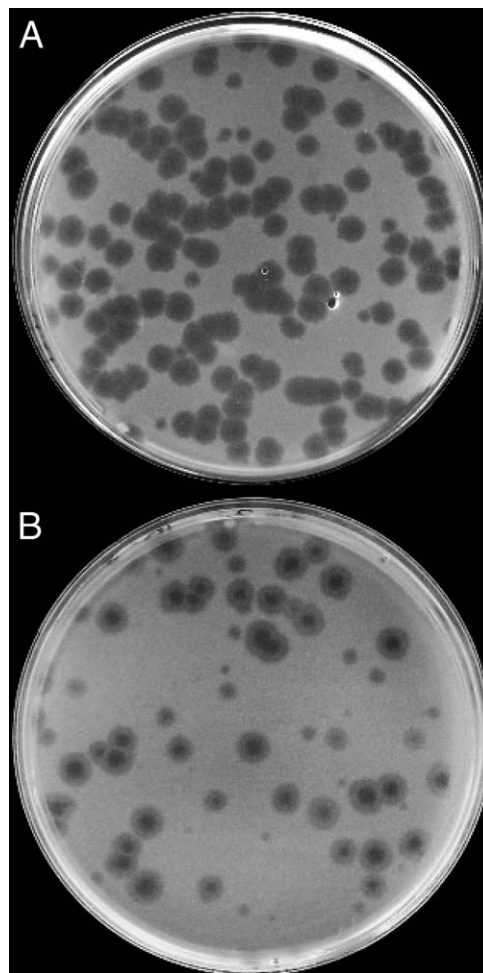


Fig. 1. Plaques of His1 (A) and His2 (B) on *Har. hispanica*. Dilutions of virus were plated with cells in 18% MGM top layer agar, poured on 18% MGM base plates, allowed to set and incubated at 30 °C (His1) or 37 °C (His2) for 48–72 h for visible plaques. Plates were 8.5 cm in diameter.

unsuccessful, although uninfected virus-resistant strains, and carrier-state (persistently infected) strains, could be readily obtained. Cells of the latter strains did not contain virus DNA in a prophage state, either chromosomally integrated or as a plasmid (data not shown).

### Stability of His2 virions

The stability of His2 and His1 under various conditions was compared and the results are summarised in Fig. 2. In high salt conditions, both viruses maintained their initial titres for at least 3 weeks when incubated at temperatures between 4 and 37 °C (data not shown). At temperatures above 50 °C, His2 lost infectivity rapidly, while His1 was slightly more resistant, losing infectivity above 60 °C (Fig. 2A). A large difference was observed in the stability of His1 and His2 to low salt: His2 lost infectivity immediately upon dilution while His1 only dropped about twofold in titre over 1 h (Fig. 2B). Both viruses were relatively stable over a wide pH range (Fig. 2C) and were sensitive to chloroform (Fig. 2D).

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