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Complete nucleotide sequence of phiCHU: A *Luz24likevirus* infecting *Pseudomonas aeruginosa* and displaying a unique host range

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Abstract

A complete nucleotide sequence of the new *Pseudomonas aeruginosa Luz24likevirus* phiCHU was obtained. This virus was shown to have a unique host range whereby it grew poorly on the standard laboratory strain PAO1, but infected 26 of 46 clinical isolates screened, and strains harboring IncP2 plasmid pMG53. It was demonstrated that phiCHU has single strand interruptions in its genome. Analysis of the phiCHU genome also suggested that recombination event(s) participated in the evolution of the leftmost portion of the genome, presumably encoding early genes.

Pseudomonas aeruginosa strains are opportunistic pathogens and a significant source of morbidity and mortality (e.g. in cystic fibrosis patients). In the wake of growing antibiotic resistance, a resurgence of interest in bacteriophage therapy has taken place to relieve the growing burden observed in healthcare systems (Burns *et al.* 2012; Weinstein *et al.* 2001).

P. aeruginosa bacteriophages (phages) are an extremely heterogeneous assemblage and in this work a novel phage of the *Luz24likevirus* genus, phiCHU, was characterized. PhiCHU was isolated from a small pond in the Moscow locality and shown to

have a unique host range; it grew poorly on the standard *P. aeruginosa* laboratory strain PAO1, but infected efficiently a number of clinical isolates resistant to other phages and lysed mucoid strains isolated from wound infections revealing itself to be a potential therapeutic agent. How it can circumvent mucoidy is currently unknown. Table S1. shows a comparative analysis of phiCHU's host range against known lytic phages phiKZ, EL, Lin68, PB1, 14/1, phiKF77, and phiKMV (Mesyanzhinov *et al.* 2002, Hertveldt *et al.* 2005, Krylov *et al.* 2007, Ceyssens *et al.* 2009, Kulakov *et al.* 1986, 1991, Lavigne *et al.* 2003). Of the 46 isolates tested phiCHU grew well on 26, but exhibited turbid growth and varying plaque morphologies on a number of other strains. Our inability to isolate lysogens from any strains led us to conclude that phiCHU behaves like a virulent phage, at least in these instances. The *Luz24likevirus* genus has previously been reported as containing lytic phages apart from PaP3 (Tan *et al.* 2007). PhiCHU's virulence and broad host range means that it is most likely suitable for use within therapeutic preparations. Importantly, phiCHU demonstrated good growth on PAO38; a *P. aeruginosa* strain containing the IncP2 plasmid pMG53. Figure S1. demonstrates the growth of phiCHU along with 8 other phages on *P. aeruginosa* strains. IncP2 plasmids confer a broad spectrum of traits to pseudomonads including, but not limited to, multiple forms of antibiotic resistance and metabolism of unusual carbon sources (Jacoby *et al.* 1983). IncP2 being the most abundant plasmids found in nosocomial strains of *P. aeruginosa* (Hanson & Olsen, 1978) were also shown to confer resistance to many phages of *P. aeruginosa* through interference in their intracellular development. This growth inhibition of different phages has been reported to be under the control of different loci within this plasmid

group (Freizon *et al.* 1989). We subsequently investigated whether pMG53 promotes efficient phiCHU growth, by conjugatively transferring this plasmid to PAO1. Upon carrying this out, PAO1 acquired sensitivity to phiCHU.

The need for phage genomics is paramount from a clinical perspective. Many phages can transfer bacterial genes by transduction, which poses a significant problem with respect to the potential dissemination of pathogenicity and resistance factors. Apt examples here would be the *P. aeruginosa* phages E79 and phiKZ (Morgan 1979, Dzhusupova 1982). Genomic analysis can help to elucidate whether these processes are likely to take place and therefore, whether a given phage is suitable for clinical use. Bacteriophage phiCHU particles were purified using isopycnic CsCl density gradient centrifugation and genomic DNA was extracted as described by Sambrook and Russell (2001). The phage was sequenced by the dideoxy method and both shotgun and primer walking on the whole genomic DNA was employed.

The genomic map of phiCHU is presented in Fig 1. phiCHU was shown to have a linear dsDNA genome of 45,626 bp with a GC content of 52.02%. 73 ORFs were predicted and annotated in Artemis (Rutherford *et al.* 2000) and functionality was assigned to 22 of these. Five ORFs encode proteins associated with genome replication, recombination and repair, 9 encode various structural proteins, 3 encode portal and terminase subunits, 2 encode the lysis machinery and the other 2 encode a putative gamma-glutamyl cyclotransferase and L-Glutamine-D-Fructose-6-Phosphate amidotransferase. All predicted genes lie in two bidirectionally transcribed units separated by a double intrinsic terminator; a structure indicative of the *Luz24likevirus* genus. Three tRNA genes

(tRNA^{Asn}, tRNA^{Asp}, and tRNA^{Pro}) were predicted which are clustered at the extreme right of the genome. The genome is delineated by 185 bp direct terminal repeats. This genomic organization and a high nucleotide homology of 94.79% to the *Luz24likevirus* vB_PaeP_C1-C14_Or (Its closest relative) (Accession: HE983844) demonstrates that phiCHU unequivocally belongs to the *Luz24likevirus* genus of the family *Podoviridae.*

Alignments of phiCHU and closely related *Luz24likeviruses* infecting *P. aeruginosa* with the Progressive Mauve algorithm (Darling *et al.* 2010) highlighted the presence of a gap of approximately 1.5 kb encompassing gp1 – gp4 (Fig. S2). This region shows a greater homology (94%) to different phages of the group (Luz24 and TL) (Ceyssens *et al.* 2008, Accession: NC_023583), which also exclusively infect *P. aeruginosa*. This finding suggests that the phiCHU genome may have evolved as a result of recombination between ancestors of vB_PaeP_C1-C14_Or and Luz24.

Knowledge of the nature of receptors utilized in phage adsorption is important for therapeutic applications. Investigations into tail fibre proteins can yield useful information with respect to variations in host range. A number of authors have implicated the presence of glycine rich regions in the identification of tail fibre proteins (Lucchini *et al.* 1999; Nilsson *et al.* 2000; Tetart *et al.* 1998). 20 of the 50 C-terminal residues of ORF 58 are glycines, thereby making this a likely candidate as one constituent of the tail fibre complex. Variations in this ORF were investigated amongst *Luz24likeviruses* and it was found that phiCHU differed by only a single

97 residue from phiMR299-2 (Alemayehu *et al.* 2012). At this point (residue 267) phiMR299-2 like other *Luz24likeviruses,* possess the
98 isoteric residues serine or threonine (Fig S3). phiCHU however, possesses proline at this point. Molecular modelling and alignment
99 of phiCHU and phiMR299-2 putative tail fibres (Fig S4) demonstrated the extent to which this substitution alters protein structure
100 and therefore, this may represent one mechanism contributing to altered host specificity of phiCHU.
101
102 It was previously demonstrated that a *P. putida* phage tf, which is distantly related to *Luz24likeviruses* has localized single-strand
103 interruptions (nicks) in its genome (Glukhov *et al.* 2012). This genomic feature was not previously demonstrated for
104 *Luz24likeviruses* infecting *P. aeruginosa*. Here it was shown that denatured phiCHU DNA produces multiple bands on agarose gels,
105 which disappeared upon ligation (Fig. S5a). The use of total genomic DNA as a template in sequencing reactions permitted the
106 localization of nicks (Fig. 1 and Fig. S5b) and allowed the identification of the consensus associated with this feature (5' –
107 TACT/RTGMC – 3'). This proved to be the same consensus as previously reported in tf (Glukhov *et al.* 2012). 14 such sites were
108 reported for tf and 15 for the *P. fluorescens* phage UFV-P2 (Eller *et al.* 2014, Glukhov *et al.* 2012). phiCHU possesses 7 instances
109 of this consensus. The purpose of this enigmatic feature in phage genomes remains unknown.
110
111 In this work, a novel member of the *Luz24likevirus* genus of phages infecting *P. aeruginosa* was isolated and characterized.
112 phiCHU was shown to possess localized single-strand interruptions in its genome. It was also found that this phage exhibited a

unique host range, whereby it grew well on PAO1 only in the presence of a plasmid from the IncP2 group. In addition, we found some evidence suggesting that recombination within disparate members of the genus contributed to the evolution of phiCHU.

Genbank Accession Number: KP233880

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Supporting Information

Additional supporting information can be found in the online version of this article:

Table S1. Sensitivity of clinical strains to phages.

Fig S1. Comparison of the sensitivity of *P. aeruginosa* strains to phages.

Fig S2. Progressive Mauve alignments of phiCHU with closely related *Luz24likeviruses*.

Fig S3. Sequence alignment of *Luz24likevirus* putative tail fibre proteins.

Fig S4. Structural alignment of phiMR299-2 and phiCHU tail fibre molecular models.

Fig S5a. Visualization of localized single-strand interruptions in phiCHU DNA**.**

Figure S5b. Localization of single-strand interruptions through sequencing on whole genomic DNA.

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Figure Legends

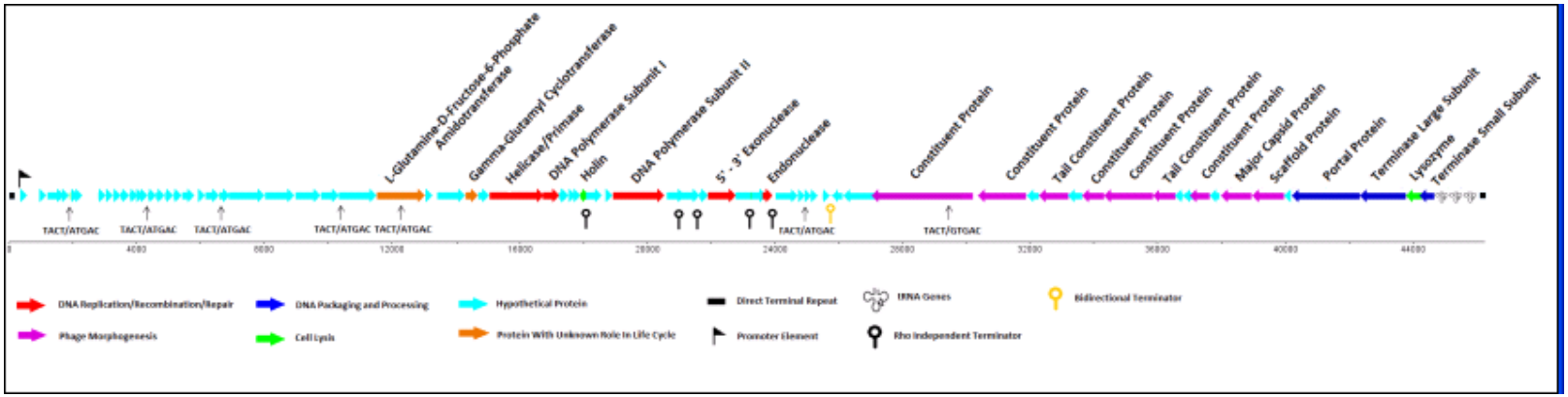

Figure 1. Genome map of the *P. aeruginosa* bacteriophage phiCHU.

Predicted ORFs are displayed as arrows indicating the direction of transcription. Functional annotations (if any) are displayed above ORFs and colour/symbol codes are presented at the bottom of the figure. Sequences associated with localized nicks are displayed at their respective positions