



This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	2009.011a,bB	(to be completed by ICTV officers)			
Short title: Create new species named Bacillus phage PBS1 to be unassigned in the family Myoviridae (e.g. 6 new species in the genus <i>Zetavirus</i>)					
Modules attached (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/>	2 <input type="checkbox"/>	3 <input checked="" type="checkbox"/>	4 <input type="checkbox"/>	5 <input type="checkbox"/>
	6 <input type="checkbox"/>	7 <input type="checkbox"/>	8 <input type="checkbox"/>	9 <input checked="" type="checkbox"/>	

Author(s) with e-mail address(es) of the proposer:

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Has this proposal has been seen and agreed by the relevant study group(s)?
Please select answer in the box on the right

N/A

ICTV-EC or Study Group comments and response of the proposer:

EC40. Move to -04

Date first submitted to ICTV:

Date of this revision (if different to above):

MODULE 2: **NEW SPECIES**

Part (a) to create and name one or more new species.

If more than one, they should be a group of related species belonging to the same genus (see Part b)

Code	2009.011aB	(assigned by ICTV officers)
To create 1 new species with the name(s): Bacillus phage PBS1		

Part (b) assigning new species to higher taxa

All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family.

Code	2009.011aB	(assigned by ICTV officers)
To assign the species listed in section 2(a) as follows:		
Genus:	unassigned	Fill in all that apply. <ul style="list-style-type: none">• If the higher taxon has yet to be created (in a later module, below) write "(new)" after its proposed name.• If no genus is specified, enter "unassigned" in the genus box.
Subfamily:	unassigned	
Family:	Myoviridae	
Order:	Caudovirales	

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
 - If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria.
 - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

PBS1 and its probable or certain relatives form a small group of 5 *Bacillus* phages with unique properties

1. PBS1 and its mutant PBS2 contain uracil instead of thymine. The presence of uracil requires a special metabolic pathway.
2. All phages have long wavy tail fibers and 'contraction fibers' which appear upon tail contraction.

MODULE 9: APPENDIX: supporting material

additional material in support of this proposal

References:

- Belyaeva NN, Azizbekyan RR. 1968. Fine structure of new *Bacillus subtilis* phage AR9 with complex morphology. *Virology* 34:176-179
- Bramucci MG, Keggin KM, Lovett PS. 1977. Bacteriophage PMB12: conversion of the sporulation defect in RNA polymerase mutants of *Bacillus subtilis*. *J Virol* 24:194-200
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- Hunter BI, Yamagishi H, Takahashi I. 1967. Molecular weight of bacteriophage PBS1 deoxyribonucleic acid. *J Virol* 1:841-842
- Rima BK, van Kleeff BHA. 1971. Similarity of *Bacillus subtilis* bacteriophages PBS1, 3NT and I10. *Antonie Leeuwenhoek* 37:265-274
- Takahashi I, Marmur J. 1963. Replacement of thymidylic acid by deoxyuridylic acid in the deoxyribonucleic acid of a transducing phage for *Bacillus subtilis*. *Nature* 197:794-795
- Vanyushin BF, Belyaeva NN, Kokurina NN, Stelmashuk VY, Tikhonenko AS. 1970. Some characteristics of uracil containing DNA from AR9 phage for *Bacillus subtilis*. *Mol Biol* 4:724-729

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders.

The use of Figures and Tables is strongly recommended.

Background

PBS1 and its potential relatives are the only DNA viruses to contain uracil instead of thymine and have unique morphological features. With respect to tail fibers, the contraction fibers of PBS1 are unique and it is difficult to see where PBS1 could have acquired them. Wavy tail fibers are found in a single giant myovirus of *B. cereus* (Bace-11, 500 nm long) (Ackermann et al., *Can J Microbiol* 41:294-297, 1995), which is profoundly different from PBS1. Although the sequence for this phage is not available, the presence of uracil instead of thymine suggests a special DNA synthesis pathway (for references see Radany et al., 1997).

A relationship between PBS1 and SPO1 has been suggested previously (Dr. Hendrix, Dr. Ackermann) in some respects, although additional experimentation (sequencing) is necessary to determine the depth of this relationship. The presence of conspicuous capsomers suggests that the capsid proteins of SPO1 and PBS1 are related. However, a distinction at the species (and presumable genus or even subfamily) level can be made, based on available literature and the comparative supporting material added below.

Further analysis of *Bacillus* phages AR9, I10, PMB12 and 3NT may warrant the establishment of a new genus.

Distinguishing Features

Uracil is substituted for thymine. Virions have three long wavy tail fibers and shorter, straight 'contraction fibers' that appear upon contraction of the tail. Phages are flagella-specific.

Virion Properties

Morphology

Capsids are icosahedra of ~113 nm in diameter and show capsomers. Extended tails measure 220 x 22 nm and have a collar, a base plate, and three terminal wavy tail fibers of about 125 x 3 nm. Contracted tails show about 20 straight 40 nm long straight fibers extending from the sheath ('contraction fibers')

Physicochemical and Chemical Properties

Particles sediment at 760 S and have a buoyant density in CsCl of 1.42 g/ml.

Nucleic Acid

The genome is 245-300 kbp (180-220 Md) in size and has 4 single-stranded gaps. Thymine has been completely replaced by uracil (A, 36.3%; U, 36.0%; G, 13.8%; C, 14.0%). PBS1 DNA is glucosylated.

Proteins	No data available.
Lipids	None known.
Carbohydrates	None known.

Genome Organization and Replication

Infecting DNA modifies host RNA polymerases to a rifampicin-resistant form.

Antigenic Properties

PBS1, PMB12, and 3NT are serologically related.

Biological Properties

Host range seems to be restricted to flagella-specific strains of *B. subtilis*. Phage PBS1 uses flagella as its primary adsorption site and then slides down to the cell wall, where the tail contracts and DNA is injected. PBS1 is a general transducing and pseudotemperate. Plaques are turbid and surviving bacteria are resistant to superinfection and release infectious virions, but the viral genome is not integrated into the host bacterium and does not persist as a plasmid. PMB12 is a sporulation-converting phage.

SUPPORTING MATERIAL

Comparison of PBS1 and SPO1

		PBS1	SPO1
Virion	Head diameter, nm	113	94
	Tail length, nm	220	150
	Collar	+	-
	3 Long wavy tail fibers	+	-
	Contraction fibers	+	-
	Sedimentation velocity, S	760	794
	Buoyant density, CsCl, g/ml	1.42	1.54
DNA	Base replacing thymine	Uracil, 36%	HMU,
	GC %	28	42
	Buoyant density, CsCl, g/ml	1.433	1.455
	Size, kbp	245-300 kb	132,562 bp
	Single-stranded gaps	4	Not known
Replication	Rifampicin sensitive	+	-
Biological:	Flagella-specific	+	-

+, Yes, present; -, no, absent.

PBS1 Bibliography

- Belyaeva NN, Azizbekyan RR. 1967. A new phage of *Bacillus subtilis* with unusual morphology (Russian). *Mikrobiologiya* 36:1054-1059
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PBS1, unusual
- Price AR, Fogt SM. 1973. Resistance of bacteriophage PBS2 infection to 6-(p-hydroxyphenylazo)-uracil, an inhibitor of *Bacillus subtilis* deoxyribonucleic acid synthesis. *J Virol* 11:338-340
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- Price AR, Frabotta M. 1972. Resistance of bacteriophage PBS2 infection to rifampicin, an inhibitor of *Bacillus subtilis* RNA synthesis. *Biochem Biophys Res Commun* 48:1578-1585
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(Lists about 20 references on uracil-DNA metabolism.)
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