RUNNING TITLE: Genome analysis of *Pseudomonas aeruginosa* phage YuA.

The genome and structural proteome of YuA, a new *Pseudomonas aeruginosa* phage resembling M6

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ABSTRACT

*Pseudomonas aeruginosa* phage YuA was isolated from a pond near Moscow. It has an elongated head, encapsulating a circularly permuted genome of 58,663 bp, and a flexible, non-contractile tail which is terminally and subterminally decorated with short fibers (*Siphoviridae*). The YuA genome is neither Mu nor λ-like, and encodes 78 gene products that cluster in three major regions involved in (1) DNA metabolism and replication, (2) host interaction and (3) phage particle formation and host lysis. At the protein level, YuA displays significant homology with phages M6, φJL001, 73, B3, DMS3 and D3112. Eighteen YuA proteins were identified as part of the phage particle by mass spectrometry analysis. Five different bacterial promoters were experimentally identified using a promoter trap assay, three of which have a σ^{54}-specific binding site and regulate transcription in the genome region involved in phage particle formation and host lysis. The dependency of these promoters for the host σ^{54} factor was confirmed by analysis in an *rpoN* mutant strain of *P. aeruginosa* PAO1. At the DNA level, YuA is 91% identical to the recently (July 2007) annotated phage M6 of the Lindberg Typing set. Despite this level of DNA homology throughout the genome, both phages combined have 15 unique genes that do not occur in the other phage. The genome organization of both phages differs substantially from the other known *Pseudomonas*-infecting *Siphoviridae*, delineating them as a distinct genus within this family.
INTRODUCTION

Despite a decade of major sequencing efforts, many aspects of the genomic diversity among bacteriophages remain to be addressed. Recent metagenomic sequencing of uncultured viral communities from oceanic regions has shown that, although common patterns of genomic organization are present, up to 90% of marine phage sequences are novel (6, 14). Other phage sequencing projects revealed distinct levels of genomic diversity for phages infecting different bacteria. For example, the diversity of phage types infecting mycobacteria (37) contrasts sharply with the dairy phages, which constitute a close-knit group (15).

In recent years, genome sequencing efforts on phages infecting *Pseudomonas aeruginosa* have revealed this group as strongly diverse at the genome organizational level, which is consistent with their reported diversity in propagation, host interaction and particle structure. The phages of *P. aeruginosa* are under investigation in scope of their therapeutic potential and to unravel the dynamic interaction with their pathogenic host. Moreover, insight in the phage genome content allows insight in evolutionary aspects of *P. aeruginosa* phages. At present, 27 complete genome sequences of phages infecting *P. aeruginosa* have been deposited in public databases (2). Among the *Siphoviruses* infecting *P. aeruginosa*, phage D3112 is probably the best studied. With the exception of a DNA modification module and a structural region coding for tail morphogenesis proteins, phage D3112 shares its overall genome organization and transposable nature with phage Mu. Its tail morphology, however, resembles the flexible tail of lambda-like particles, in contrast to Mu-like particles which have a rigid contractile tail (49). This mosaicism is relatively common among temperate phages and suggests horizontal
evolution. Phage B3 is another transposable *P. aeruginosa* infecting phage, but more
distantly related to phage Mu compared to phage D3112 (12). Phage DMS3 shares DNA
similarity with phage D3112 and is able to transduce DNA between *P. aeruginosa* strains
PA14 and PAO1 (17). Phage D3 resembles phage λ both from an organizational and
morphological point of view (25).

Here, we report the characterization of the new *P. aeruginosa* phage YuA on a
morphological, genomic and proteomic level. Phage YuA was isolated in a pond near
Moscow and belongs to the *Siphoviridae* family. Morphological data suggest relatedness
to phage M6 from the Lindberg typing set (1, 28). Both YuA and M6 are significantly
different from other *Pseudomonas*-infecting *Siphoviruses* deposited in the public
databases. Therefore, an in-depth analysis of the phage YuA genome sequence and the
particle protein content was performed.

**MATERIALS AND METHODS**

**Bacteriophage isolation, bacterial strains, media and plasmids.** Bacteriophage YuA
was isolated out of an environmental water sample, using standard enrichment methods
(3). The host bacterium *P. aeruginosa* PAO1 and 73 other AFLP-typed clinical *P.
aeruginosa* strains were kindly provided by J.P. Pirnay (38). The *P. aeruginosa* PAO1
*rpoN* mutant strain (*rpoN::tet*) (7) was kindly provided by H. Arai. Bacterial strains were
grown in standard Luria-Bertani medium. Electrocompetent *P. aeruginosa* PAO1 cells
were prepared by six consecutive wash steps of an overnight 2 ml culture with 1 ml of
ice-cold ultra-pure water, and finally resuspending in 50 µl H_2O.
**Phage purification and electron microscopic imaging.** High-titer stocks of YuA were obtained by an overnight incubation of $10^6$ plaque forming units (PFU) in the presence of *P. aeruginosa* PAO1 cells using the standard soft agar overlay technique (3). Lawns of soft agar were collected and resuspended in 20 ml of phage buffer (150 mM NaCl, 10 mM MgSO$_4$, 10 mM Tris.HCl pH 8) and briefly vortexed. Phage particles were collected by centrifugation (20 min; 4,000 x g), concentrated in presence of polyethylene glycol 8,000 (8%, wt/vol) and purified by two successive rounds of CsCl density gradient centrifugation. Purified phage particles were negatively stained with uranyl acetate (2%, wt/vol) and visualized by transmission electron microscopy.

**DNA isolation, characterization and sequencing.** Phage DNA was isolated as described elsewhere (35). Restriction digests were performed according to the manufacturer’s protocol. Initial sequence data was obtained from a shotgun library of phage DNA in pUC18. Several consecutive rounds of primer walking were performed directly on phage DNA, until the sequence assembled into a single contig with an average fourfold redundancy. Open reading frames were predicted using Genemark HMM (31) and visually inspected for the presence of convincing ribosome binding sites. Translated ORF sequences were compared with known proteins using BLASTP (5) and PSI-BLAST (4) algorithms against the non-redundant GenBank protein database. In addition, smaller, non-predicted ORFs which are conserved between YuA and M6 were considered as functional ORFs, based on tBLASTx comparisons between both phage genomes. Prokaryotic promoters were predicted using the BDGP (39) and SAK (22) prediction programs, and by scanning the genome for conserved intergenic motifs using the MEME/MAST algorithm (8). Putative terminators were searched using Transterm (19).
and transmembrane helices were detected using the TMHMM algorithm (33). Finally, tRNA genes were searched by using the tRNAscan-SE program (30).

**Experimental promoter identification.** Purified YuA DNA was randomly sheared by sonication. Fragments ranging from 200 to 400 bp were recovered from agarose gel, end-repaired, phosphorylated and ligated into the *SmaI*-digested and dephosphorylated vector pTZ110, a promoterless broad-host-range vector with a *lacZ* operon fusion (44). A threefold redundant promoter library was obtained after electroporation of the ligation mixture into freshly prepared electrocompetent *P. aeruginosa* PAO1 cells (1.8 kV, 25 µF, 250 Ω) and plating onto LB plates supplemented with 125 µg/ml carbenicillin and 40 µg/ml X-gal. Plasmid DNA from blue colonies was isolated by the alkaline lysis method (41) and inserts were sequenced using a vector-specific primer (5′-GCCACCTGACGTCTAAGAAAAC-3′). Specific β-galactosidase activity of these selected clones was confirmed and quantified in liquid cultures (32).

**Mass spectrometry.** For identification of structural YuA proteins, 10 µl of concentrated phage solution (10^{11} PFU) was reduced in 2 mM β-mercaptoethanol, heat-denatured (95°C, 5 min) and loaded on a standard 12% SDS-PAGE gel. The entire lane was cut into slices which were subjected to trypsin digestion (46). For the alternative Whole-Phage Shotgun Approach (WSA), an equal amount of phages was destabilized by four successive rounds of freeze-thawing and sonication, heated for 10 min at 95°C and reduced in the presence of 10 mM DDT for 1 h at 56°C. Disulfide bounds were blocked by alkylation with 10 mM Iodoacetamide, followed by an overnight trypsinisation of the whole reaction mixture at 37°C. Peptides generated by the two methods were separated...
by liquid chromatography with a linear 5-60% (vol/vol) acetonitrile gradient and subsequently identified using ESI-MS/MS as described previously (27).

**Nucleotide sequence accession number.** The genome of bacteriophage YuA was deposited at Genbank under accession number AM749441.

**RESULTS AND DISCUSSION**

**General features of phage YuA and its genome.** Bacteriophage YuA propagates on *P. aeruginosa* PAO1 and infects 13 out of 73 diverse *P. aeruginosa* strains from a worldwide collected and ALFP-typed library. YuA displays a small (1 mm diameter) and turbid plaque morphology, suggesting a temperate nature. The phage does not infect *P. putida* and *P. fluorescens* or other gram-negatives like *E. coli*, *Shigella* and *Salmonella* species, but is able to lyse *Burholderia solanacearum* at low efficiency (EOP 10⁻⁷).

Electron microscopic imaging revealed YuA as a typical member of the *Siphoviridae* family of dsDNA bacteriophages (Caudovirales), having a flexible, non-contractile tail (Figure 1). In contrast to the well-known *Pseudomonas Siphoviridae* phages D3, B3 and D3112 that resemble phage λ, phage YuA has an elongated head (B2 morphotype) resembling *P. aeruginosa* phage M6 (1). The phage YuA head size is ~72 x 51 nm and the tail length is ~145 nm. Besides an elongated head, both YuA and M6 have striated tails which are terminally and subterminally decorated with short fibers. Phage M6 is reported to be morphologically identical to *Xanthomonas oryzae* phage XP12 (1), and shown to adsorb to non-retractile host pili (11).

YuA genomic DNA is insensitive to the activity of 10 out of 13 tested restriction enzymes, including many common enzymes like *Hind*III, *Bgl*II and *Eco*RI, and the
methylation-dependent \textit{DpnI} (GA\textsuperscript{m}/TC). Given the sensitivity towards digestion with \textit{Sau3A} (/GATC) and the methylation-sensitive \textit{SmaI} (C\textsuperscript{+}CC\textsuperscript{+}/GGG), it can be concluded that YuA contains unmethylated adenine and cytosine residues. This is in contrast with the morphologically related phage XP12, which is known to contain a 5-methylcytosine instead of cytosine in its genome (20). \textit{In silico} analysis revealed the absence of 9 out of 10 recognition sites, although four \textit{EcoRI}-sites are present despite the insensitivity towards the restriction enzyme. Furthermore, it became clear during genome sequencing that isolated YuA DNA is rather inaccessible to standard PCR amplification using various primers, annealing temperatures and commercially available DNA polymerases. These observations suggest the presence of another base substitution or modification, as is discussed below. Resistance towards restriction during phage infection could also be provided by gene product 45 (gp45) of YuA, which displays high similarity with the \textit{ArdB} antirestriction protein (pfam e-value of 10\textsuperscript{-30}). This plasmid-encoded protein inhibits efficiently restriction by members of the three known families of type I restriction endonucleases (10).

\textbf{YuA genome sequence and similarity to other phages.} The genome of YuA comprises 58,663 bp and has a G+C content of 64.3 \%, strongly resembling the G+C average (65\%) of its host. In total, 78 open reading frames (ORFs 1 to 77 and ORF 60.1) were predicted on the circular genome map (Figure 2, supplementary data table S1), all oriented in the same direction and leaving only 4\% of the YuA genome as non-coding. No tRNA genes were predicted. The genome of YuA is neither \textit{Mu} nor \textit{\lambda}-like and can be roughly divided in three functional regions, containing gene products involved in (1) nucleotide
metabolism and DNA replication, (2) host interaction and (3) particle structure, packaging and host lysis (Figure 2).

The YuA genome displays an overall 91% DNA similarity to phage M6 (NC_007809) (26), present throughout the entire genome and resulting in >80% amino acid identity in 92% of the predicted ORFs. The data obtained for YuA confirm the recent gene predictions made for M6 in GenBank. Only six genome regions contain unique YuA or M6 sequences, accounting in total for 15 differential gene products, four of which occur in YuA and eleven in M6. Further comparative analysis between YuA and M6 also revealed YuA gp70 and gp71 as most deviating in the structural region, hinting at a role of these proteins in host recognition and surface adhesion (Figure 3).

Comparison to other phages reveals significant similarity of 29 predicted YuA proteins to proteins encoded by φJL001 (63,469 bp), a phage that infects an uncharacterized marine α-proteobacterium JL001. Phage φJL001 is reported as a temperate phage, shares roughly the YuA particle morphology and appears to lack the ability to form stable lysogens (29). In addition, eighteen particle structure-related YuA proteins share amino similarity to gene products of P. aeruginosa phage 73 from the Lindberg typing set (28). Despite major similarity in head morphogenesis genes, phage 73 is morphologically identical to phage D3112 (1), and does not show the elongated head morphology typical for YuA, M6 and φJL001 particles. This might be explained by the smaller genome content of phage 73 (42,999 bp) compared to the 60 kb-genomes of the latter phages. Finally, eight YuA proteins (gp70 to gp77) which are most probably involved in host attachment and interaction, share sequence similarity with the transposable and pilus-specific P. aeruginosa phages B3, DMS3 and D3112 (Figure 2 and supplementary table S1). The
latter gene products also appear in several bacterial genome sequences, as prophage or
cryptic phage elements in e.g. *Hahella chejuensis*, *Xylella fastidiosa*, *Burkholderia
cepacia* and *Haemophilus ducreyi* and in *Burkholderia cepacia* phage BcepNazgul
(NC_005091, 57,455 bp).

The choice of the YuA genome sequence zero point was based on genome comparisons
with phages φJL001, D3112, DMS3, B3, predicted gene functions and promoter
prediction/identification in phage YuA. The YuA zero point differs from the phage M6
zero point, which might be reconsidered for consistency among these related phages.

**Regulatory elements.** Motif searches lead to the identification of two different
conserved intergenic motifs that could be involved in the transcription regulation of
phage YuA (Figure 2). To experimentally identify host promoter sequences, promoter
activity was determined quantitatively in *P. aeruginosa* cells by measuring the β-
galactosidase activity of individual clones of the constructed promoter trap library (32, 44). Five YuA regions were identified from which transcription of the vector-encoded
*lacZ* gene was initiated by the *P. aeruginosa* transcriptional machinery (Figure 4). Two
different promoter types were distinguished based on sequence information and promoter
strength. The first type, found in front of genes 2 and 50, has a clear σ^70^-like consensus
sequence (TTAGGT-N_17^-TtaAAT) and yields 1,021 Miller Units (MU) of β-
galactosidase activity. The second promoter type is located in the genome region
involved in particle formation and host lysis. It precedes genes 55, 58 and 68, and
displays approximately a twofold activity (2,152 MU) compared to the first promoter.
Conserved GG and GC elements in this second promoter type are separated by a DNA
stretch corresponding to one helical turn, resembling σ^54^-binding sites (9). This finding
was further investigated using an *rpoN* deletion mutant of *P. aeruginosa* PAO1, which is unable to produce the σ^{54} transcription factor (7). Infection studies (MOI 0.1-10^8) revealed the inability of YuA to replicate inside this mutant. In a second step, five pTZ110 constructs containing the identified promoter regions (Figure 4) were electroporated into this mutant and the transformants were assayed for β-galactosidase activity. In contrast to the two identified σ^{70} promoters (P_2 and P_{50}) which largely maintained their activity, transcription of the reporter β-galactosidase gene downstream the putative σ^{54} promoters was absent (Figure 4). This proves that the three promoters preceding genes 55, 58 and 68 specifically require the σ^{54} binding factor to initiate transcription of the downstream genes. This is, to our knowledge, the first time that this is demonstrated for a bacteriophage, and implies the dependency of YuA on additional transcriptional factors (enhancer-binding proteins) to initiate RNA synthesis of the late phage proteins (34).

No promoter activity could be associated with a third, perfectly conserved intergenic motif (CTTTACTTACTTCGG-N9-TATACTT) preceding genes 8, 12, 28, 42 and 45 (Figure 2), which might indicate the need for one or more phage-encoded protein(s) to control the expression of these YuA genes. Alternatively, another bacterial-encoded transcription factor that is only present under certain circumstances might be required to initiate transcription.

In addition, four ρ-independent terminators were predicted, downregulating transcription beyond genes 5 (unknown function), 12 (predicted DNA repair enzyme), 55 (structural protein) and 56 (major capsid protein), respectively (Figure 2).
Gene products involved in DNA metabolism and genome replication. The YuA genome region from ORF2 to ORF23 encodes several proteins which are predicted to be involved in nucleotide metabolism (gps 17, 22 and 23) and DNA replication (gps 7, 13, 21 and 41). Gene 22 encodes a putative ribonucleotide reductase which catalyses the committed step to DNA synthesis by reduction of ribonucleoside diphosphates (rNDPs) to the corresponding deoxyribonucleoside diphosphates (dNDPs). Deoxycytidylate (dCMP) deaminase (gp23) catalyzes the deamination of deoxycytidylate to deoxyuridylate (dUMP). Interestingly, a dUMP hydroxymethylase (dUMP-HMase) function is predicted for gp17. In this enzymatic reaction, dUMP serves as substrate for the addition of a hydroxymethyl group, using \( \text{CH}_2\text{H}_4\text{folate} \) as a cofactor and generating the modified base hydroxymethyl-deoxyuridylate (43). The presence of this modified base was shown in *Bacillus* phage SPO1 (50) and is also predicted for φJL001 and M6. dUMP-HMases share similarity with the more widespread and highly conserved group of thymidylate synthases, which use the same cofactor in the reduction of dUMP to dTMP. Sequence alignment of YuA gp17 with dCMP hydroxymethylase of T4 and the corresponding gp17 homologous proteins in φJL001 and SPO1 (Figure S1 in the Supplementary data section) clearly shows conservation of catalytically important residues (Glu60, Cys148 and Asp179), deoxyribose-binding residues (His216 and Tyr218) and phosphate-binding residues (Lys28, Arg123, Arg124, Arg168 and Ser169) (47).

Host interaction. The YuA genome region bearing genes with predicted functions in host interaction ranges from ORF25 to ORF45. Unique to YuA is the presence of a diguanylate-cyclase or GGDEF domain (ORF 44), which is widespread in bacterial
proteins, functioning as a global second messenger controlling motility and adhesion in bacterial cells (21). The putative repressor (gp25) contains helix-turn-helix motifs similar to those of the phage λ repressor, while the predicted integrase (gp26) differs significantly from the two major families of tyrosine and serine site-specific recombinases (23). Thus far, we have not been able to isolate a stable lysogenic P. aeruginosa PAO1 strain. Our assays involved isolation of phage-resistant P. aeruginosa PAO1 clones from turbid plaques and analysis for the presence of phage sequences by PCR and DNA restriction analysis. Neither stably integrated, nor stably nonintegrated phage genome sequences could be detected. Similar results were reported for phage φJL001 (29) and Vibrio parahaemolyticus phages VP16T and VP16C (45). Strikingly, the YuA integrase exhibits 32% amino acid identity with the integrase of Vibriophages VP5 and VP2, which share similarity with VP16T and VP16C integrases. Apparently, these vibriophage-like integrase proteins are also unrelated to the well-studied tyrosine or serine recombinase families and exhibit distinct integrase behaviour. The YuA integrase may require specific – not yet determined – physiological conditions or a different host strain for stable lysogenic establishment.

**The structural proteome.** ESI-MS/MS analysis of gel-separated phage particle proteins led to the experimental identification of 16 predicted proteins, reaching sequence coverages up to 66.1% (Table 1). The two most abundant proteins were identified as gp56 and gp66, suggesting a function as major capsid and tail proteins. The abundance of these two proteins was confirmed by denaturation and subsequent MS analysis of whole phage particles, as described in the materials and methods section. The latter method allowed detection of additional peptides which were not found in the first analysis, thus
increasing the overall coverage. Moreover, two additional YuA proteins (gp64 and gp75) were experimentally confirmed and identified as part of the virion particle (Table 1). These results roughly delimit the YuA genome region involved in particle formation and host release to genes 52 up to 77 and corroborate the complementarity of the two MS approaches. It also supports our previous finding that the WSA approach is well suited to trace small or less abundant phage proteins (27), and it provides an experimental annotation of the particle proteins, accounting for 23% of the predicted ORFs.

**The YuA lysis cassette.** Bacteriophage YuA encodes a delineated cluster of four overlapping lysis genes as typically observed in lambdoid phages, in P22-like phages and the more closely related phage B3 (51). The YuA lysis cassette is located within the genome region encoding phage particle proteins, presumably between the head and tail morphogenesis genes. Compared to the organization of the lysis cassette in lambdoid phages, the YuA genes within this cassette are rearranged since the putative endopeptidase $R_z$ (gp60) and the embedded $R_z1$ (gp60.1) (reading frame +1) genes precede the holin (gp61) and endolysin (gp62) gene (16, 52). YuA $R_z$ and $R_z1$ proteins share similarity with the M6 gene products gp21 and gp21.1 (newly predicted based on homology to the YuA $R_z1$ protein), respectively. $R_z1$ has a typically high proline content (10/66) and is predicted as a lipoprotein (LipoP 1.0, fatty acid lipidation at Cys15). With the exception of phage M6 gp22, YuA gp61 does not show homology to known proteins. However, its position in the putative lysis cassette, its small size and three transmembrane domains with an N-terminal inside topology strongly suggest a function as a class I holin (48). Gp62 (the predicted endolysin) has a putative signal peptide sequence (SignalP 3.0, P=0.951) with a cleavage site at position 23 (ALA-QD). The
presence of a signal peptide and lytic activity of gp62 is supported by the observation of
cellular toxicity of recombinantly expressed gp62 in absence of sodium azide, an
inhibitor of the Sec secretion complex. Expression in presence of sodium azide largely
elevates toxicity of gp62 expression (unpublished results). The presence of a signal
peptide sequence suggests the need for an inhibitory mechanism of the exported lysins to
prevent early lysis of the host cells. Recent research showed that full-activity of the
previously exported fOg44 lysin is only achieved after sudden, ion-unspecific dissipation
of the proton motive force, an event undertaken by the holin (36, 42). In this way, holins
still function as a time regulator of lysis.

GENERAL CONCLUSIONS
With the estimated ten million tailed-phage species in the environment (39), it is doubtful
that we will ever reach the point at which sequencing more phage genomes will fail to
add significantly to our understanding of phage diversity and evolution. It is compelling
however that, despite evident genetic mosaicism, a limited (but steadily increasing)
number of phage genera can be delineated, like the M6-like Siphoviridae investigated in
this study. At this point, it remains unclear whether this clustering is an artifact of current
sampling, or that these groups actually exist (18). Nevertheless, the sequencing of phage
YuA supports the previously stated hypothesis that local viral diversity seems to be high,
whereas global diversity is relatively low because of movement of viruses between
environments (13).

One must also consider the fact that research which is limited to genome sequencing
generates vast numbers of genes with unknown functions in public databases, and
functional studies involving non-model phages are lagging far behind. With well-chosen
experimental studies, these gaps can be (partially) closed quite efficiently. For example, the experimental identification of 18 YuA particle proteins (Table 1) allows tentative functional annotations of their corresponding proteins in respective phage particles of phages M6, φJL001, D3112, B3, DMS3, 73, BcepGomr, BcepNazgul, XP15, SETP3 and KS7. Novel studies unraveling unknown gene functions can give valuable insights in diversity of lifestyle strategies, and should be encouraged.

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**FIGURE LEGENDS**

Figure 1. Electron microscopic image of phage YuA particles. The scale bar represents 100 nm. Phage YuA has an elongated head and a flexible tail.

Figure 2. Circular representation of the YuA genome. The outer circle represents the YuA ORFs, with indication of their predicted functions involved in DNA metabolism and replication, host interaction, particle formation and host lysis. Experimentally confirmed structural proteins are marked with an asterisk, confirmed phage promoters are indicated as black arrows. Predicted (non-confirmed) promoters and terminators are shown as open arrows and stem-loop structures, respectively. The inner circles represent similar ORF regions of phages 73 (purple), φJL001 (blue) and B3 and D3112 (red). Corresponding e-values are indicated for φJL001, B3 and D3112.

Figure 3. Pair-wise comparison of bacteriophages YuA and M6. The predicted open reading frames and their mutual amino acid identity are indicated in red (>90%), orange (>80%) and yellow (>50%). ORFs unique to YuA (4) and M6 (11) are hatched and purple, respectively, and predicted functions are indicated. Nucleotide identity throughout both genomes is illustrated by the middle graph, comparing both phages ORF by ORF using a sliding window of 60 bp.

Figure 4. Promoter assay of the YuA fragment library. Five promoter regions were identified and are named after the gene they precede. Measurements of β-galactosidase activity in wild-type *P. aeruginosa* PAO1 and the rpoN mutant are indicated in black and grey, respectively. Data represent the average and standard deviation of six samples per promoter construct for accurate quantification of the promoter activity by the Miller assay.
FIGURE 3

DNA Metabolism & replication

Host Interaction

Particle formation & host lysis

YuA

M6

% DNA Identity

0 10 20 30 40 50 60 70 80 90 100

gp17 [u/L001] e = 4.0E-10

ORF071 [FS] e = 2.6E-2
FIGURE 4

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Table 1. Identification of the structural proteins of YuA.

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<th>1-DE approach</th>
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