

Combined Antibacterial Activity of Phage Lytic Proteins Holin and Lysin from *Streptococcus suis* Bacteriophage SMP

Yibo Shi · Ning Li · Yaxian Yan · Hengan Wang ·
Yan Li · Chengping Lu · Jianhe Sun

Received: 21 January 2012 / Accepted: 16 March 2012 / Published online: 17 April 2012
© Springer Science+Business Media, LLC 2012

Abstract Development of novel antibacterial agents is required to control infection with multidrug-resistant *Streptococcus suis*. HolSMP and LySMP, the holin and lysin of *S. suis* serotype 2 bacteriophage, named SMP, are responsible for lysis of host cells and release of progeny phage. HolSMP and LySMP expressed in *Escherichia coli* BL21(DE3) exerted efficient activity at 37 °C, pH 5.2, with addition of 0.8 % β -mercaptoethanol. Lytic spectra of purified HolSMP, LySMP or HolSMP + LySMP mixture were investigated. HolSMP, exhibiting a narrow lytic spectrum, was effective against *Staphylococcus aureus* and *Bacillus subtilis*, which were insensitive to LySMP. Moreover, HolSMP was identified as a promising antibacterial agent which was able to extend the spectrum of LySMP. The data suggest that combined use of holin and lysin could be a candidate strategy for resolution of drug resistance.

Introduction

Overuse of antibiotics has led to an increase in the worldwide prevalence of antibiotic-resistant bacteria [6, 14, 19, 23]. *S. suis* is an important zoonotic pathogen that leads to economic losses and severe invasive diseases in humans, with meningitis being the most common clinical presentation [4, 12, 21]. It has been reported that 421 isolates of *S. suis* from clinically healthy sows in China exhibited high level resistance to commonly used antibiotics, including tetracycline, sulfisoxazole, clindamycin, erythromycin and trimethoprim/sulfamethoxazole [10, 26, 28]. Novel antibiotics can be developed, but the pathogens ultimately become resistant to such drugs. To break this vicious cycle, phage therapy has been recommended, including the use of phage products, as a drug-independent strategy to control bacterial infection [15].

For most bacteriophages, lysis of the host requires at least two proteins: lysin and holin [11]. Lysins are phage-encoded proteins and act to degrade the bacterial host cell wall before the release of phage, and have been reported as the best example of phage antimicrobial agents. Indeed, the utilization of phage lysins as enzybiotics is a hot research topic [8, 18]. Holins are small phage-encoded proteins that accumulate in the cytoplasmic membrane during the period of late-protein synthesis after infection and assist lysins to complete lysis of the host cell [9]. In this respect, holins have the potential to be used as therapeutic agents in their own right [7]. Moreover, the antibacterial activities of several holin-like proteins, such as the *tmp1* gene encoded protein identified from a goat skin surface metagenome and BhlA protein of *B. licheniformis*, have been reported over past decade [1, 22]. However, the combining antibacterial activity of holin with lysin was not well studied.

Electronic supplementary material The online version of this article (doi:10.1007/s00284-012-0119-2) contains supplementary material, which is available to authorized users.

Y. Shi · Y. Yan (✉) · H. Wang · J. Sun (✉)
Department of Animal Science, School of Agriculture and
Biology, Shanghai Jiao Tong University, Shanghai Key
Laboratory of Veterinary Biotechnology, Shanghai, People's
Republic of China
e-mail: yanyaxian@sjtu.edu.cn

J. Sun
e-mail: sunjhe@sjtu.edu.cn

N. Li · Y. Li · C. Lu
Key Laboratory of Animal Disease Diagnostic and Immunology,
Ministry of Agriculture, Nanjing Agricultural University,
Nanjing, People's Republic of China

SMP is a *S. suis* serotype 2 (SS2) lytic bacteriophage isolated from nasal swabs of healthy Bama minipigs [13]. Streptococcal strains SS2-4, SS2-6, and SS2-H are sensitive in plaque assays [16, 27]. LySMP is the endolysin of SMP, and shows efficient degradation of *S. suis* treated with phenylmethanesulfonyl fluoride (PMSF) or lysozyme at 37 °C and pH 5.2, and exhibited a broader spectrum of activity than whole phage against bacteria investigated [27]. Moreover, LySMP almost completely disperses the biofilms formed by *S. suis* [16].

Here, we aimed to characterize the antibacterial activity of HolSMP, the holin of SMP, and determine the synergistic antimicrobial activity of HolSMP and LySMP against *S. suis*, as the next step towards developing novel therapeutic agents against bacterial infection.

Materials and Methods

Bacterial Strains, Plasmids, Bacteriophages and Growth Media

S. suis SS2, SS7, and SS9 strains were isolated from diseased pigs between 1998 and 2005 in China. *Streptococcus equi* ssp. *zooepidemicus* (SEZ) reference strain ATCC35246, *S. aureus* reference strain ATCC25923 and *B. subtilis* reference strain ATCC6633 were purchased from the American Type Culture Collection (ATCC). *Salmonella enterica* strain ATSEL0114 and *E. coli* strain MC1061 were donated by Professor Lu of Nanjing Agricultural University. *E. coli* strains DH5 α and BL21(DE3) were purchased from Tiangen Biotech (Beijing, China). All of the *S. suis* strains and SEZ were cultured in Todd–Hewitt broth (THB) or agar medium, supplemented with 2 % (v/v) newborn bovine serum, at 37 °C, while the other strains were cultured in Luria–Bertani (LB) broth. Plasmid pET-28a(+) (Novagen) and pET-lys containing the *LySMP* gene was propagated in strain BL21 in LB broth containing 50 μ g/ml kanamycin, as described previously [27]. SMP is an *S. suis* serotype 2 lytic bacteriophage first isolated by Ma et al. from nasal swabs of healthy Bama minipigs [13]. Bacteriophages were prepared as described previously [27].

DNA Extraction and Plasmids Construction

DNA was isolated from SMP as described previously [27]. According to the sequence of SMP (GenBank accession no. EF116926), primers were designed to amplify the *HolSMP* gene by PCR (forward primer: GCGGAATTCatggttatgatgttat; reverse primer: ATCCTCGAGGCTTTGAT-TAGTTtca; new restriction sites *Eco*RI and *Xho*I were indicated by shadow, and lowercase letters represented

sequences corresponding to that of *HolSMP*) from the purified phage genomic DNA. The amplified holin gene was then cloned into the pET-28a(+) prokaryotic expression vector using the *Eco*RI and *Xho*I restriction sites incorporated into the PCR primers, designated as pET-hol. The ligation product was transformed into competent *E. coli* DH5 α cells. Plasmids were extracted from positive transformants and sequenced.

Expression Analysis of HolSMP and LySMP

The recombinant plasmids pET-lys and pET-hol were transformed into competent *E. coli* BL21(DE3) cells. Overnight grown culture of BL21(DE3) containing plasmid pET-lys or pET-hol was diluted 1:100 and incubated to OD₆₀₀ 0.5–0.6. Protein expression was induced by addition of isopropyl- β -thiogalactopyranoside (IPTG) to a final concentration of 1 mM and shaking at 30 °C at 150 rpm for 4 h. The growth of clones after induction was monitored by measuring OD₆₀₀.

Purification of HolSMP and LySMP

Protein expression was induced as described above. To obtain *E. coli* lysates containing HolSMP, the pellet of 1-L-induced culture was resuspended in 15 ml of lysis buffer (50 mM sodium phosphate, pH 8.0, 300 mM NaCl). Detergent-soluble material on cytoplasmic membrane was extracted as previously described, except that the detergent was replaced with dialyzable detergent *N*-octyl- β -D-glucopyranoside (OGP) [24]. The recombinant HolSMP in solution with detergent was purified using nickel-affinity chromatography. All of the buffers used in purification contained 1 % OGP. LySMP was prepared according to the method of Wang [27]. Then, the purified proteins were dialyzed and concentrated as described previously [24]. Concentrations of HolSMP and LySMP were estimated [25].

SDS-PAGE and Zymography

Purified HolSMP or LySMP was subjected to SDS-PAGE at 80 V in a 5 % polyacrylamide gel gradient and then at 120 V in a 12 % gradient. Protein bands were detected by dyeing with Coomassie brilliant blue R250. Zymography was performed as previously described with *S. suis* SS2-H cells as an indicator [27]. IPTG-induced BL21 cells containing pET-28a(+) were used as a control.

Characterization of HolSMP Activity

For turbidity reduction assay, *B. subtilis* was used as an indicator. Culture was grown to mid-log phase (OD₆₀₀

0.5), collected and resuspended in buffers at specific pH to OD₆₀₀ 1.0. A 100 µl of purified HolSMP with a final concentration of 100 µg/ml and 100 µl indicator cells were mixed and added to 96-well plates. Plates were incubated for 30 min. Changes in OD₆₀₀ were recorded. The effect of pH on HolSMP bactericidal activity was determined in several buffers (20 mM sodium acetate, pH 4.0, 20 mM sodium acetate, pH 5.2, 20 mM PBS, pH 6.8, 10 mM PBS, pH 7.2, 20 mM Tris-HCl, pH 8.5). Likewise, the ability of HolSMP at different temperatures (4, 17, 27, 37, and 42 °C) and the optimum concentration of β-mercaptoethanol (0, 0.5, 0.8, 1, 3, and 5 %) for refolding of protein were also tested.

Antibacterial Activity of Phage Lytic Proteins

Antibacterial activities of HolSMP, LySMP or HolSMP + LySMP mixture were tested by addition of purified proteins to a well in an agar plate overlaid by indicator cells. Twenty-three strains of SS2, SS7, SS9, SEZ, *S. aureus*, *E. coli*, and *S. enterica* were investigated. Cultures were grown to mid-log phase (OD₆₀₀ 0.8), collected and resuspended in PBS (pH 5.2) at 1 % of the original culture volume. The bacterial pellet was washed three times with PBS. THB agar plates containing a 1 % inoculum of indicator *Streptococcus* strain were prepared. Likewise, *S. aureus*, *E. coli* and *S. enterica* were inoculated in LB agar or THB agar plates. Four wells with a diameter of 0.5 cm were cut into the agar, and 20 µl purified HolSMP (with 20 µl PBS and 0.8 % of β-mercaptoethanol), LySMP (with 20 µl PBS and 0.8 % β-mercaptoethanol), mixture of HolSMP and LySMP (20 µl respectively and 0.8 % β-mercaptoethanol) and 40 µl PBS with 0.8 % of β-mercaptoethanol as a control were added to these wells, respectively. All experiments were performed in triplicate. After incubation for 5 h at 37 °C, clear halos around the wells indicated antibacterial activity.

Results

Expression, Purification of HolSMP and LySMP

Recombinant phage proteins, HolSMP and LySMP, were expressed in host strain *E. coli* BL21(DE3) in LB broth containing ampicillin by addition of IPTG. The proteins were purified, dialyzed, concentrated and checked for antibacterial activity. SDS-PAGE analysis was performed to examine the purified target proteins. Bands of approximately 16 and 55 kDa were HolSMP and LySMP, respectively (Fig. 1 lane 1 and Fig. 2a lane 1). In the zymogram of autoclaved cells of SS2-H as the substrate, LySMP showed highly efficient degradation of the cell

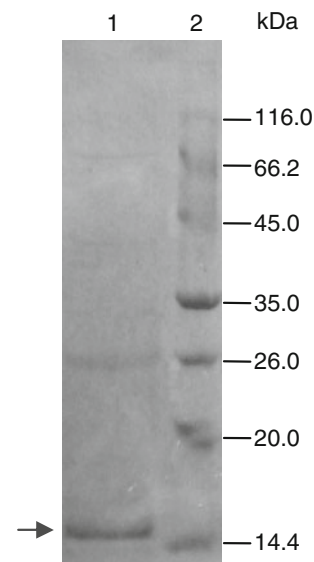


Fig. 1 SDS-PAGE analysis of purified HolSMP. Purified HolSMP was pointed by the arrow (lane 1), and the marker was shown on lane 2

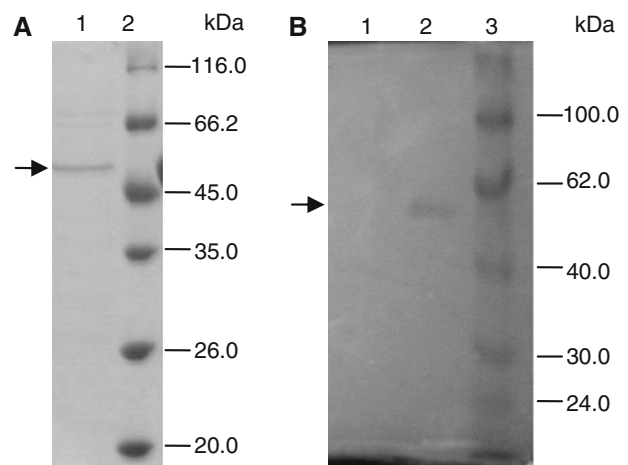


Fig. 2 SDS-PAGE and Zymogram analysis of LySMP. **a** SDS-PAGE of purified LySMP. The purified LySMP was pointed by the arrow (lane 1), and marker was shown on lane 2. **b** Zymogram analysis of LySMP. *E. coli* BL21(DE3) harboring pET-28a(+) (lane 1) was performed as a negative control of *E. coli* BL21(DE3) harboring pET-hol(+) (lane 2). The lytic activity of LySMP appeared as a translucent band pointed by arrow on the opaque background. The marker was shown on lane 3

wall (Fig. 2b lane 2). *E. coli* BL21(DE3) harboring pET-28a(+) (Fig. 2b lane 1) was performed as a negative control.

Characterization of HolSMP

Employing the turbidity reduction assay, the optimum pH, temperature conditions and concentration of β-mercaptoethanol

for the holin were determined. Greatest enzymatic activity was obtained at pH 5.2 (Fig. 3a). Turbidity decrease was high at pH 8.5 in comparison to pH 7.2, presumably due to autolytic activity of bacteria in an alkaline background. A temperature profile for HolSMP activity was also determined. The bacterial cells were most susceptible to HolSMP at 37 °C (Fig. 3b). The decrease in turbidity was relatively high by addition of 0.5–1 % β -mercaptoethanol (Fig. 3c).

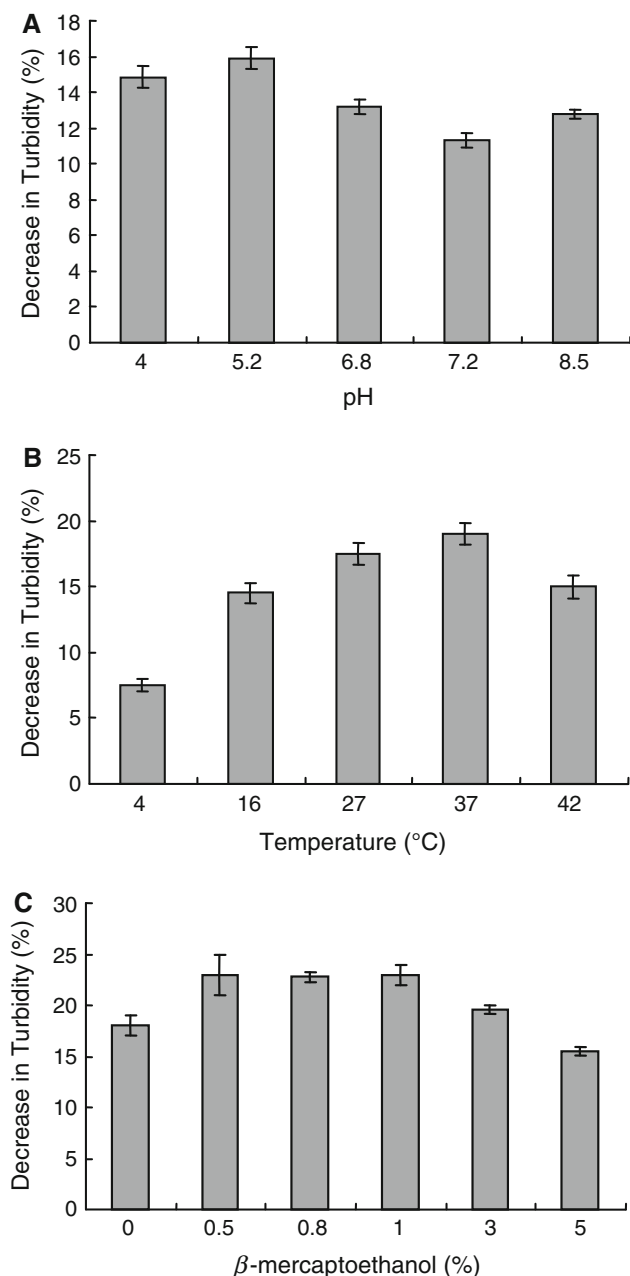


Fig. 3 Characterization of HolSMP activity. Percent reduction in the turbidity of *B. subtilis* after treatment with 10 μ g of HolSMP for 30 min. **a** pH profile of HolSMP antibacterial activity. **b** Temperature profile of HolSMP antibacterial activity. **c** β -Mercaptoethanol profile of HolSMP antibacterial activity. Values represent mean \pm SD

Antibacterial Activity of HolSMP and Its Combination with LySMP

Antibacterial activities were tested by addition of purified proteins into wells in agar plates overlaid by indicator cells (Fig. 4). Twenty-four different cell substrates, including 19 clinical isolates of *S. suis*, an SEZ strain, an *S. aureus* strain, an *E. coli* strain, an *S. enterica* strain and a *B. subtilis* strain were used as indicators. Only *S. aureus* and *B. subtilis* HolSMP were sensitive to HolSMP (Table 1). Five out of 17 strains of SS2 could be lysed by LySMP, as well as SS7 and SS9. However, 10 more strains of SS2 and a strain of *S. enterica* were sensitive to the mixture of HolSMP and LySMP. Combined usage of HolSMP with LySMP exhibited an extensive spectrum of lytic activity against the bacteria investigated.

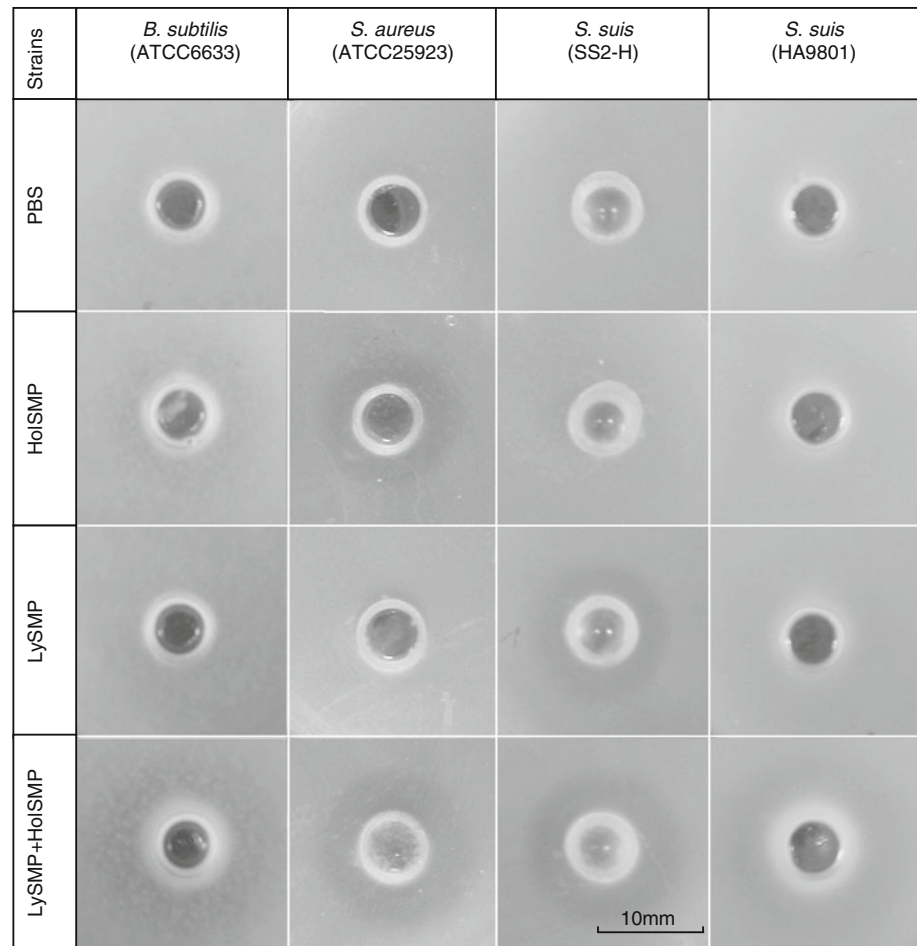
Discussion

Phage antimicrobial agents, using phage themselves or their products, have recently been proposed for resolution of antibiotic resistance. In recent years, the application of living phages, non-replicating, genetically modified phages, lysins, and “protein antibiotics” has been reported [2], but the practical application of the first two has been limited to a narrow host spectrum and has several safety issues.

In this study, we found that HolSMP, the holin protein, could be a novel antimicrobial agent. Holins are small phage-encoded proteins with a different lytic mechanism from that of lysins. During progress of progeny phage release, holins accumulate non-specifically and form lesions in the cytoplasmic membrane, while lysins target and degrade bacterial murein. When applied exogenously, we found that HolSMP was effective against *S. aureus* and was able to kill the cells. The holin-like protein from *B. licheniformis* has also been reported consistently to be effective at killing several types of Gram-positive bacteria, such as methicillin-resistant *S. aureus* (MRSA) and *Micrococcus luteus* [1]. Compared with *Streptococcus* spp., *S. enterica* and *E. coli*, many *S. aureus* strains have no capsule but a slime layer, so it is possible that it might be easier for holin to target the membrane of *S. aureus*, but further study is needed to establish the exact antimicrobial mechanism of holins.

Phage-encoded lysins, which could degrade peptidoglycan of Gram-positive bacteria exogenously, are considered to be new and potent weapons against drug-resistant pathogens. Lysins against a number of Gram-positive pathogens, such as *B. anthracis* and *Streptococcus pyogenes*, were reported [3, 17]. The engineered lysin ClyS, constructed by fusing the catalytic domain and a unique

Fig. 4 Antibacterial activity of HolSMP and LySMP. Purified HolSMP and LySMP were added into the wells of agar plates. The results of *B. subtilis*, *S. aureus*, *S. suis* (SS2-H and HA9801) were presented as prototypes. After incubation, a clear halo around the well indicated lysis



binding domain from two different phages infecting *Staphylococcus*, was considered as a prototype of the research on improvement of lytic efficiency of lysins. ClyS showed synergistic effect with vancomycin and oxacillin in treatment of *S. aureus* infection as well [5]. Moreover, the antibodies developed after repeated ClyS exposure did not inhibit ClyS activity [20]. These findings implied a bright future of lysins as antibacterial agents.

Our research focused on the combined antibacterial activity of holin and lysin. A previous study on LySMP has reported that 15 out of 17 strains of *S. suis* SS2 treated with PMSF could be lysed, as well as strains SS7 and SS9, SEZ and *S. aureus*. In particular, LySMP can efficiently disrupt biofilms formed by *S. suis*. In the present study, two SS2 strains 05-465 and ZY05722, which were insensible to SMP, were lysed by LySMP. These results suggested that lysin exhibited a larger bacteriolytic spectrum than its corresponding phage. Nonetheless, SS2 strains, such as HA05729-1, SS2-9, and SS-N, were insensible or less sensitive to the LySMP under “physiological” conditions. Therefore, it was also a major issue to improve the availability of LySMP. Although the simple HolSMP only exhibited a narrow lytic spectrum, it could lyse two strains

of *S. aureus* and *B. subtilis*, which were insensible to LySMP. Moreover, 12 more strains, including strains of an *S. aureus*, *B. subtilis* strain and *S. enterica* were lysed by a mixture of HolSMP and LySMP. HolSMP showed synergistic activity with LySMP. It was encouraging that both HolSMP and LySMP had greatest activity at 37 °C and pH 5.2 with 0.8 % β -mercaptoethanol. The average body temperature of mammals is 37 °C, and the pH of oral cavity, saliva, nasal cavity and vagina is 4.0–5.5. On the one hand, this could facilitate in vivo animal tests. On the other hand, the simultaneous administration of HolSMP and LySMP is feasible. Therefore, the combined application of LySMP and HolSMP to control *S. suis* infection has potential.

To improve the activity and facilitate purification of HolSMP, an oligohistidine tag was fused to HolSMP. We found that such an addition at one terminus fundamentally changed the character of HolSMP, altered the triggering time and weakened its lesion-forming activity (Fig. 5). Further research suggested that insertion of an oligohistidine tag after codon 139 yielded HolSMP that showed essentially normal timing and lysis profiles (Fig. 6), which might be helpful for preparation and application of

Table 1 Bacterial strains testing for antibacterial activity

Strain	Description	Serotype	Results of cells ^a			Plaque assay
			HolSMP	LySMP	HolSMP + LySMP	
SS2-9	<i>S. suis</i>	2	–	–	+	–
HA9801	<i>S. suis</i>	2	–	–	+	–
HA9802	<i>S. suis</i>	2	–	–	+	–
05-465	<i>S. suis</i>	2	–	+	++	–
15	<i>S. suis</i>	2	–	–	+	–
19-2(A)	<i>S. suis</i>	2	–	–	+	–
SS2-4	<i>S. suis</i>	2	–	+	++	+
C166	<i>S. suis</i>	2	–	–	–	–
zy05719	<i>S. suis</i>	2	–	–	–	–
006731	<i>S. suis</i>	2	–	–	–	–
HA05729-1	<i>S. suis</i>	2	–	–	+	–
ZY05722	<i>S. suis</i>	2	–	+	++	–
SS2-H	<i>S. suis</i>	2	–	+	+	+
13	<i>S. suis</i>	2	–	–	–	–
SS2-6	<i>S. suis</i>	2	–	+	++	+
SS-N	<i>S. suis</i>	2	–	–	+	–
2-4	<i>S. suis</i>	2	–	–	++	–
SH040805	<i>S. suis</i>	7	–	+	++	–
SH040817	<i>S. suis</i>	9	–	+	++	–
ATCC35246	SEZ		–	–	–	–
ATCC25923	<i>S. aureus</i>		+	–	+	–
ATSEL0114	<i>S. enteria</i>		–	–	+	–
MC1061	<i>E. coli</i>		–	–	–	–
ATCC6633	<i>B. subtilis</i>		+	–	+	–

^a ++ strong intensity, + weak intensity, – absence of lysis (no clearing lytic zone)

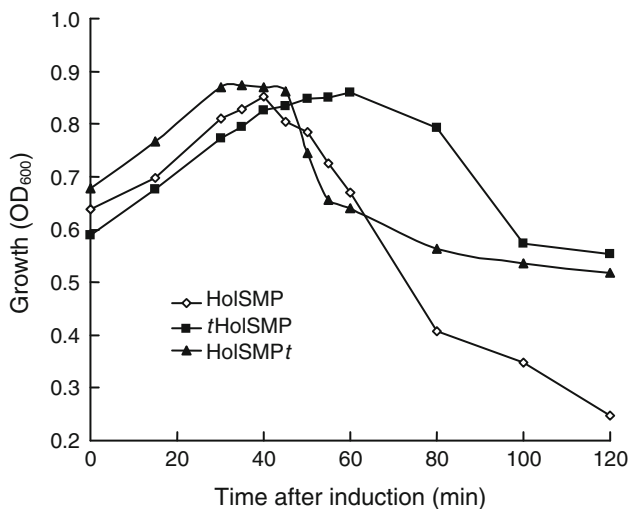


Fig. 5 Lysis profiles of BL21(DE3)pLysS harboring plasmids bearing the *HolSMP* gene with inserts encoding an oligohistidine tag at either the N or C terminus. The oligohistidine tag was indicated by *t*. Thus, the N-terminally tagged *HolSMP* allele and the C-terminally tagged one were defined as *tHolSMP* and *HolSMPt*, respectively

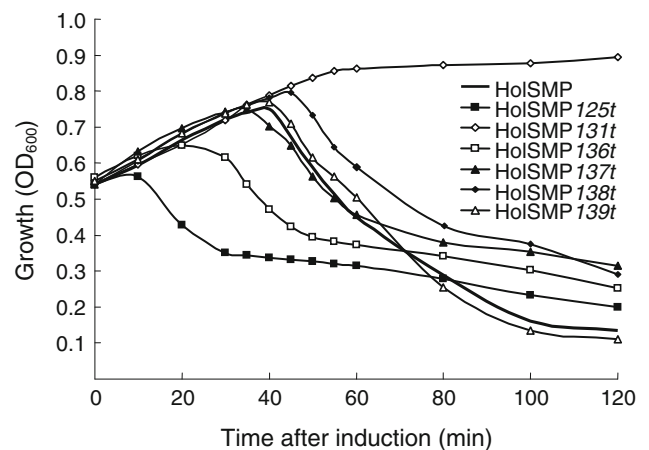


Fig. 6 Lysis profiles of BL21(DE3)pLysS harboring plasmids bearing the *HolSMP* gene with inserts encoding an oligohistidine tag after codon 125, 131, 136, 137, 138, 139. The oligohistidine tag was indicated by *t*. For example, the *HolSMP* with oligohistidine tag behind codon 125 was defined as *HolSMP125t*

HolSMP in the future. The plasmids, primers, and strategies of plasmids construction used on oligohistidine-tag-encoding insertions were given in Online Resource 1.

The combined application of HolSMP and LySMP proved that holin and lysin had a synergistic effect when administered exogenously. Further investigation of combined usage of phage antibacterial agents and a candidate strategy for solution of drug resistance is now warranted.

Acknowledgments We thank Alan McCarthy of the University of Liverpool for providing constructive suggestions. The research was supported by the National Natural Science Foundation of China (31172381) and fund from State Key Laboratory of Veterinary Etiological Biology (SKLVEB2010KFKT004).

References

- Anthony T, Chellappa GS, Rajesh T, Gunasekaran P (2010) Functional analysis of a putative holin-like peptide-coding gene in the genome of *Bacillus licheniformis* AnBa9. Arch Microbiol 192:51–56
- Bernhardt TG, Wang IN, Struck DK, Young R (2002) Breaking free: “protein antibiotics” and phage lysis. Res Microbiol 153:493–501
- Bourguet FA, Souza BE, Hinz AK, Coleman MA, Jackson PJ (2012) Characterization of a novel lytic protein encoded by the *Bacillus cereus* E33L *ampD* gene as a *Bacillus anthracis* antimicrobial protein. Appl Environ Microbiol. doi:10.1128/AEM.06906-11
- Chang B, Wada A, Ikebe T, Ohnishi M, Mita K, Endo M, Matsuo H, Asatuma Y, Kuramoto S, Sekiguchi H, Yamazaki M, Yoshikawa H, Watabe N, Yamada H, Kurita S, Imai Y, Watanabe H (2006) Characteristics of *Streptococcus suis* isolated from patients in Japan. Jpn J Infect Dis 59:397–399
- Daniel A, Euler C, Collin M, Chahales P, Gorelick KJ, Fischetti VA (2010) Synergism between a novel chimeric lysin and oxacillin protects against infection by methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 54:1603–1612
- Daoud Z, Kourani M, Saab R, Nader MA, Hajjar M (2011) Resistance of *Streptococcus pneumoniae* isolated from Lebanese patients between 2005 and 2009. Rev Esp Quimioter 24:84–90
- Donovan DM (2007) Bacteriophage and peptidoglycan degrading enzymes with antimicrobial applications. Recent Pat Biotechnol 1:113–122
- Fischetti VA (2010) Bacteriophage endolysins: a novel anti-infective to control Gram-positive pathogens. Int J Med Microbiol 300:357–362
- Gründling A, Manson MD, Young R (2001) Holins kill without warning. Proc Natl Acad Sci USA 98:9348–9352
- Hendriksen RS, Mevius DJ, Schroeter A, Teale C, Jouy E, Butaye P, Franco A, Utinane A, Amado A, Moreno M, Greko C, Stärk KD, Berghold C, Myllyniemi AL, Hoszowski A, Sunde M, Arestrup FM (2008) Occurrence of antimicrobial resistance among bacterial pathogens and indicator bacteria in pigs in different European countries from year 2002–2004: the ARBAO-II study. Acta Vet Scand 50:19
- Krupović M, Daugelavičius R, Bamford DH (2007) A novel lysis system in PM2, a lipid-containing marine double-stranded DNA bacteriophage. Mol Microbiol 64:1635–1648
- Lun ZR, Wang QP, Chen XG, Li AX, Zhu XQ (2007) *Streptococcus suis*: an emerging zoonotic pathogen. Lancet Infect Dis 7:201–209
- Ma YL, Lu CP (2008) Isolation and identification of a bacteriophage capable of infecting *Streptococcus suis* type 2 strains. Vet Microbiol 132:340–347
- Markowska-Daniel I, Urbaniak K, Stepniewska K, Pejsak Z (2010) Antibiotic susceptibility of bacteria isolated from respiratory tract of pigs in Poland between 2004 and 2008. Pol J Vet Sci 13:29–36
- Matsuzaki S, Rashel M, Uchiyama J, Sakurai S, Ujihara T, Kuroda M, Ikeuchi M, Tani T, Fujieda M, Wakiguchi H, Imai S (2005) Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. J Infect Chemother 11:211–219
- Meng XP, Shi YB, Ji WH, Zhang J, Wang HA, Lu CP, Sun JH, Yan YX (2011) Application of a bacteriophage lysin to disrupt biofilms formed by the animal pathogen, *Streptococcus suis*. Appl Environ Microbiol 77:8272–8279
- Nelson D, Loomis L, Fischetti VA (2001) Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme. Proc Natl Acad Sci USA 98:4107–4112
- O’Flaherty S, Ross RP, Coffey A (2009) Bacteriophage and their lysins for elimination of infectious bacteria. FEMS Microbiol Rev 33:801–819
- Onanuga A, Temedie TC (2011) Nasal carriage of multi-drug resistant *Staphylococcus aureus* in healthy inhabitants of Amassoma in Niger delta region of Nigeria. Afr Health Sci 11:176–181
- Pastagia M, Euler C, Chahales P, Fuentes-Duculan J, Krueger JG, Fischetti VA (2011) A novel chimeric lysin shows superiority to mupirocin for skin decolonization of methicillin-resistant and -sensitive *Staphylococcus aureus* strains. Antimicrob Agents Chemother 55:738–744
- Princivalli MS, Palmieri C, Magi G, Vignaroli C, Manzin A, Camporese A, Barocci S, Magistrali C, Facinelli B (2009) Genetic diversity of *Streptococcus suis* clinical isolates from pigs and humans in Italy (2003–2007). Euro Surveill 14(33):1–7
- Rajesh T, Anthony T, Saranya S, Pushpam PL, Gunasekaran P (2011) Functional characterization of a new holin-like antibacterial protein coding gene *tmp1* from goat skin surface metagenome. Appl Microbiol Biotechnol 89:1061–1073
- Sass A, Marchbank A, Tullis E, Lipuma JJ, Mahenthalingam E (2011) Spontaneous and evolutionary changes in the antibiotic resistance of *Burkholderia cenocepacia* observed by global gene expression analysis. BMC Genomics 12:373
- Smith DL, Chang CY, Young R (1998) The lambda holin accumulates beyond the lethal triggering concentration under hyper-expression conditions. Gene Expr 7(1):39–52
- Smith DL, Struck DK, Scholtz JM, Young R (1998) Purification and biochemical characterization of the lambda holin. J Bacteriol 180(9):2531–2540
- Vela AI, Moreno MA, Cebolla JA, González S, Latre MV, Domínguez L, Fernández-Garayzábal JF (2005) Antimicrobial susceptibility of clinical strains of *Streptococcus suis* isolated from pigs in Spain. Vet Microbiol 105:143–147
- Wang Y, Sun JH, Lu CP (2009) Purified recombinant phage lysin LySMP: an extensive spectrum of lytic activity for swine streptococci. Curr Microbiol 58:609–615
- Zhang C, Ning Y, Zhang Z, Song L, Qiu H, Gao H (2008) In vitro antimicrobial susceptibility of *Streptococcus suis* strains isolated from clinically healthy sows in China. Vet Microbiol 131:386–392