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S. Sable, Sylvie S. Lortal

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MINI-REVIEW

S. Sable · S. Lortal

The lysins of bacteriophages infecting lactic acid bacteria

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Abstract This short review highlights the complete absence of literature on lysins of bacteriophages infecting species like *S. salivarius* subsp. *thermophilus*, *Pediococcus* and *Leuconostoc* species, *L. helveticus*, *L. acidophilus*, *L. plantarum* and *L. brevis*, which are also widely used in the dairy industry. The lysins described share some similar biochemical characteristics: optimal pH and temperature, site of hydrolysis inside the peptidoglycan, and some activators and inhibitors. The cloning of the genes encoding these lysins only began in the last few years and four of them have been completely sequenced. In the future, these lysin genes could be interestingly compared to the host autolysin(s) gene(s). By contrast, the passage of phage lysins through the cytoplasmic membrane of the host cell in order to reach the peptidoglycan (via a signal sequence or the presence of a holin) seems not to be clearly resolved. The presence of a second open-reading frame upstream from the gene of the lysin, enabling a putative holin to be encoded, has already been suggested. No doubt our ever increasing knowledge about bacteriophage genome organization will help to elucidate this question. Meanwhile the obtention of a *Lactococcus* strain with an autolytic phenotype, using a bacteriophage lysin gene, as well as the successful use of purified PL1 lysin to obtain protoplasts of *L. casei* encourage us to continue to explore the field of bacteriophage lysins.

Introduction

Lactic acid bacteria are widely used in a variety of dairy fermentation processes. They contribute to the flavour, the texture and the protection of the product from spoilage microorganisms. Nevertheless, they are susceptible to bacteriophage infections, as described for the first time in 1935 (Whitehead and Cox 1935). Since that time, numerous reports have described the morphology (Accolas and Spillman 1979a, b; Sechaud et al. 1988; Sechaud 1990), the genome (Teuber and Lembke 1983; Mata et al. 1986; Jarvis et al. 1991) and the adsorption on the bacterial cell (Yokokura 1977; Ishibashi et al. 1982; De Vos et al. 1984; Valyasevi et al. 1990) of these bacteriophages. Resistance mechanisms of bacteria have received particular attention (Chopin et al. 1984; Watanabe et al. 1984b; Gautier and Chopin 1987; Klaenhammer 1987; Hill et al. 1990) in order to construct bacteriophage-resistant starters for the dairy industry (Sanders et al. 1986).

For temperate as well as for virulent bacteriophages, the release of native bacteriophage particles implies a hydrolysis step of the bacterial cell wall by a lytic enzyme known as the bacteriophage lysin. This lysin is probably also implicated in the injection of the DNA phage into the cell. This key enzyme of the bacteriophage propagation has been extensively studied in some gram-positive bacteria like *B. subtilis* (Ward et al. 1982; Garvey et al. 1986) and *S. pneumoniae* (Garcia et al. 1987; Romero et al. 1990a) and in the gram-negative bacteria *E. coli* (Young 1992). Paradoxically the lysins produced by the bacteriophages infecting lactic acid bacteria have received little attention. The purification and biochemical characterization of these enzymes, and a better knowledge of their regulation, would perhaps contribute to stopping or to slowing down their lytic action in industrial conditions. In addition, these enzymes, once they have been partially purified, could be used to hydrolyse the cell wall of some lactic acid bacteria, resistant to hen egg-white

S. Sable
Université La Rochelle, Laboratoire de Microbiologie appliquée;
Avenue Marillac, 17042 La Rochelle cedex 01, France

S. Lortal (✉)
INRA, Laboratoire de Recherches de Technologie Laitière,
65 rue de St. Brieuc, 35042 Rennes cedex, France.
Fax: + 33-99.28.53.50

Table 1 Lysins of bacteriophages infecting lactic acid bacteria

Species	Phage	Lysin	Molecular mass estimation (Da)		Optimum of pH and temperature	Specificity	References
			By SDS-PAGE	From the gene sequence			
<i>Streptococcus lactis</i>	(ϕ v) ML3	ML3			6.5–6.8/37°C	Muramidase	Reiter and Oram 1963
	C10				6.5–6.8/37°C		Oram and Reiter 1965
	C2		(7S)		6.0–6.5/—	—	Tourville and Johnstone 1966
	C10						Tourville and Tokuda 1967
<i>Lactococcus lactis</i> subsp. <i>cremoris</i>	ϕ C2(w)	C2(w)	46 000		6.5–6.9/37°C	Muramidase	Mullan and Crawford 1985a, b
	ϕ vML3	ML3	24 000	21 090	—	—	Shearman et al. 1989, 1991, 1992
	C2	C2		22 000	—	—	Ward et al. 1993
<i>Lactococcus lactis</i> subsp. <i>casei</i>	ϕ US3	LytA	29 000	28 977	—	Amidase?	Platteuw and De Vos 1992
	PL-1	PL-1	30 000	—	6.0–6.5/45°C	—	Watanabe et al. 1984a
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>			37 000	—	—	Muramidase	Hayashida et al. 1987
	ϕ MV1	LysA	24 000	21 120	—	Muramidase?	Boizet et al. 1990

lysozyme, in order to obtain protoplasts or to fragilize the cells efficiently. Moreover, the construction of strains with an "autolytic" phenotype with the gene encoding the bacteriophage lysin (Heinrich and Plapp 1984), as suggested by Shearman et al. (1989) for *Lactococcus* species, implies the characterization of the gene and of the enzyme itself. Such strains, releasing their intracellular enzymes more efficiently (peptidases, proteases, lipases; all or some of them possibly amplified in the future) in the curd, could be useful in a strategy of accelerated ripening.

Finally, in the course of a more general view of lytic enzymes, Garcia et al. (1987) and Romero et al. (1990b) have suggested an evolutionary relationship between the bacteriophage lysins and the autolysins of the host cells. This relationship would be interesting to observe in lactic acid bacteria also, even if for the moment no autolysins have actually been purified in these microorganisms.

The aim of this review is to summarize the available literature on the lysins of bacteriophages infecting lactic acid bacteria.

The lysins of bacteriophages infecting *Lactococcus* species

Several lysins of bacteriophages infecting the species *Lactococcus lactis* (previously named *Streptococcus lactis*; Schleifer et al. 1985) have been described (Table 1). Four of them [C2, C10, ML3, C2(w)] have been partially purified directly from cell lysates by ammonium sulphate precipitation generally followed by chromatographic steps (Reiter and Oram 1963; Oram and Reiter 1965; Tourville and Johnstone 1966; Tourville and Tokuda 1967; Mullan and Crawford 1985a, b). The purified enzymes showed similar values for optimal pH and temperature, as well as some similar activators and inhibitors (Table 2). Moreover, the lysins ML3 and C2(w) were found to be muramidases (cleaving the β 1-4 N-acetylmuramic-acid–N-acetylglucosamine linkage in the peptidoglycan (EC 50.1.2.3.17). They were all more sensitive to heat when compared to the phage producer; on the other hand they were less strain-specific, as demonstrated by their lytic spectra (Table 3). Indeed several strains of group D streptococci and also strains of *L. lactis*, all resistant to the bacteriophage, were sensitive to the partially purified lysins. It could be noticed that C10 lysin was also active against *Micrococcus luteus*. Some authors have emphasized the importance of this lytic spectrum in a mixed starter system (Naylor and Czulak 1956; Mullan and Crawford 1985b). They demonstrated that the lactic acid production could be perturbed by a phage lysin even if some strains constituting the mixed starter were resistant to the bacteriophage itself. These authors also suggested the use of these partially purified lysins to obtain protoplasts of *L. lactis*, in particular for strains resistant to

Table 2 Heat denaturation, activators and inhibitors of the partially purified lysins

Lysins	Inactivation by heating	Activators	Inhibitors	References
ML3, C10	$T > 37^{\circ}\text{C}$	Na^+ (0.15 M), Li^+ , NH_4^+ , Ca^{2+} , Mg^{2+}	$\text{Na}^+ > 0.15 \text{ M}$	Oram and Reiter 1965
C2, C10	$56^{\circ}\text{C}/20 \text{ min}$	NaCl , LiCl , CaCl_2	<i>p</i> -Chloromercuribenzoate (0.02 mM) iodoacetamide (0.01 M)	Tourville and Tokuda 1967
C2(W)	$47^{\circ}\text{C}/5 \text{ min}$ $57^{\circ}\text{C}/1 \text{ min}$	Na^+ (0.1 M), K^+ (0.1 M) Mn^{2+} (3 mM), Ca^{2+} (5 mM), Co^{2+} (8 mM), Mg^{2+} (5 mM)	$\text{Na}^+ > 1 \text{ M}$, $\text{K}^+ > 1 \text{ M}$ Co^{2+} (30 mM) <i>p</i> -chloromercuribenzoate (0.01 mM) glutathione (0.06 M) urea (1 M)	Mullan and Crawford 1985a, b
PL1	—	—	Cu^{2+} (1 mM), Fe^{3+} (1 mM), Cd^{2+} (1 mM) monoiodoacetate (1 mM) <i>p</i> -chloromercuribenzoate (10 mM) diisopropylfluorophosphate (1 mM) phenylmethylsulphonylfluoride (10 mM) <i>O</i> -phenanthroline (0.1 mM)	Watanabe et al. 1984

hen egg-white lysozyme. For that purpose, conditions affecting the lysis of group N streptococci by the purified $\phi\text{C2(w)}$ lysin were determined (carbon source, growth temperature, lyophilisation of the cells) (Mullan and Crawford 1985c). In a similar manner, the partially purified lysin of the bacteriophage ϕvML3 has been used to liberate cell wall proteinases in lactic streptococci (Thomas et al. 1974).

The gene encoding the lysin of ϕvML3 has been recently cloned using an *E. coli*/bacteriophage λ host vector system (Shearman et al. 1989). The sequence of the gene corresponded to a protein of 187 amino acids with a deduced molecular mass of 21090 Da; this was coherent with the 24 kDa protein expressed by *E. coli* (as determined by sodium dodecyl sulphate/polyacrylamide gel electrophoresis, SDS-PAGE). The ML3 lysin was active on a wide range of *Lactococcus* strains but lacked homology with known lytic enzymes. Interestingly, significant homology was shown with protein 15 of the bacteriophage PZA infecting *B. subtilis*, which is involved in phage morphogenesis. Thus, the ML3 lysin could possibly fulfil a second function in phage maturation. The ML3 lysin gene has been located on the restriction map of the ϕvML3 genome in the central region (Shearman et al. 1991). Moreover, attempts to clone it in *L. lactis* have recently been successful (Shearman et al. 1992) leading to a strain with an "autolytic" phenotype. The lysis occurred only after the exponential growth phase and the strain can now be tested in a flavour-acceleration strategy for cheese ripening.

Recently the bacteriophage c2 lysin was expressed in *E. coli* in an identical manner to the ML3 lysin (Ward et al. 1993). The DNA sequence obtained was very similar, with a number of silent substitutions, and an apparent deletion altering the C terminus of the pro-

tein. In this work, a codon-usage table for the lysin genes of several bacteriophages was also provided.

On the other hand, the gene (*lytA*) encoding the lysin (LytA) of the bacteriophage ϕUS3 has been cloned and expressed in *E. coli* (Patteuw and De Vos 1992). The nucleotide sequence of *lytA* showed an open-reading frame of 774 bp corresponding to a protein of 258 amino acids with a deduced molecular mass of 28977 Da; this was in agreement with the 29 kDa protein expressed by *E. coli*. Interestingly, the amino acid sequence of LytA was similar to that of the autolysin of *S. pneumoniae*. This fact suggests that the lysin could be an amidase, the first one among the lysins already described (Table 1).

The lysins of bacteriophages infecting *Lactobacillus* species

In the case of the dairy lactobacilli, the lysin of the phage PL1 infecting *Lactobacillus casei* is the only one to be partially purified directly from a cell lysate (Watanabe et al. 1984a; Hayashida et al. 1987). The optimum pH, inhibitors and specificity were similar to those of the lysins of the bacteriophages infecting *Lactococcus lactis* (Tables 1, 2). The lytic spectrum has been tested on very few species and the lysin seems to be specific to the host strain, and to the *L. casei* species (Table 3). In addition, the N-terminal sequence of the purified PL1 lysin was determined up to 27 amino acid residues (AY?INKEFALGANEG?KZVAN?LYIIL). Interestingly, the PL1 lysin has been used to obtain protoplasts of *L. casei* (Watanabe et al. 1987) and these protoplasts were recently well transfected by PL1 phage DNA (Watanabe et al. 1992). In 1986, the genome of the virulent LL-H phage infecting *L. lactis*

Table 3 Lytic spectra of the isolated lysins; the number of strains tested is shown in parentheses

Species	Lysins								
	ML3	C10 ^a	C2	C10 ^b	C2(w)	LL-H ^c	LytA ^c	PL1	<i>Leuconostoc</i> lysin
Group N streptococci			+	+					
<i>Lactococcus cremoris</i>	+ (5)	+ (5)			+ (8)		+ (12)		— (1)
<i>lactis</i>	+ (3)	+ (3)			+ (6)		+ (22) — (1)		— (1)
<i>diacetylactis</i>	+ (1)	+ (1)			+ (2)				
<i>Streptococcus thermophilus</i>					— (2)				— (1)
Group D streptococci									
<i>Streptococcus bovis</i>	+ (1)	+ (1)			+ (2)				
<i>zymogenes</i>	+ (1)	+ (1)							
<i>durans</i>	+ (1)	+ (1)			+ (2)				
<i>liquefaciens</i>	+ (1)	+ (1)							
<i>faecalis</i>	+ (1)	+ (1)			+ (1)				
<i>faecium</i>					+ (1)				
Group A streptococci									
<i>Streptococcus pyogenes</i>	— (3)	— (3)						— (1)	
<i>mutans</i>								— (4)	
Group B streptococci									
<i>Streptococcus agalactiae</i>	— (3)	— (3)							
Group C streptococci									
<i>Streptococcus dysgalactiae</i>	— (3)	— (3)			— (2)				
<i>Lactobacillus casei</i>								+ (2)/ — (2)	
<i>arabinosus</i>						—			
(<i>plantarum</i>)								— (1)	— (1)
<i>bulgaricus</i>					—	+ (6) — (4)			
<i>fermentum</i>					—				
<i>lactis</i>						— (4)			
<i>helveticus</i>						+ (3) — (3)			
<i>Leuconostoc lactis</i>					— (2)				+ (1)
<i>dextranicum</i>					— (2)				+ (11) — (4)
<i>cremoris</i>					— (2)				+ (6) — (1)
<i>mesenteroides</i>									+ (4) — (1)
<i>Micrococcus luteus</i>	—			+	—				
<i>E. coli</i>				—	—				— (1)
<i>Staphylococcus aureus</i>									
<i>Bacillus megaterium</i>									
<i>Bacillus subtilis</i>						+			
<i>Aerobacter</i> sp.				—					
<i>Klebsiella</i> sp.				—					
<i>Sarcina lutea</i>				—					

^a Oram and Reiter 1965^b Tourville and Tokuda 1967^c Lysin expressed by *E. coli* after cloning

was characterized and the gene coding for the phage lysin cloned in *E. coli* (Trautwetter et al. 1986). The LL-H lysin expressed by *E. coli* showed a large lytic spectrum (Table 3) since it was able to lyse *B. subtilis*.

Recently another gene (*lysA*) encoding the lysin of the temperature bacteriophage mv1 infecting *L. delbrueckii* subsp. *bulgaricus* has been sequenced after cloning in *E. coli* (Boizet et al. 1990). The molecular mass of the deduced protein was 21120 Da. This was coherent with the apparent molecular mass of the lytic protein expressed by *E. coli* (24 kDa). Moreover, the

lysin produced by *E. coli* was active on some strains of *L. delbrueckii* subsp. *bulgaricus*, but also on strains of *L. helveticus* and *S. salivarius* subsp. *thermophilus*. No lytic effect was detected on *Lactococcus* strains. Because of the significant homology between the N-terminal regions of the LysA lysin, the muramidase of Chalaropsis (Flech et al. 1975) and the muramidase of the Cp1 phage infecting *Streptococcus pneumoniae* (García et al. 1988), Boizet et al. have suggested that LysA was also a muramidase. It could be noticed that no homology was noted between the N-terminal

sequence of LysA and that of PL-1, which was shown to be a muramidase by biochemical assays (Table 1). In addition, no homology was observed between the complete amino acid sequences of ML3 or LytA (Table 1) and that of LysA. Taking into account the absence of characteristic promoter sequences and the presence of an open-reading frame with unknown function upstream from the *lysA* gene, Boizet et al. (1990) suggested that this gene was part of an operon. This was substantiated by the genome analysis of another temperate bacteriophage (mv4), closely related to the bacteriophages mv1 and LL-H (Mata et al. 1986), and infecting *L. delbrueckii* subsp. *bulgaricus*; the operon implicated in the cell lysis (*lysB-lysA*) was located on the restriction map of the mv4 genome (Lahbib-Mansais et al. 1992).

The lysins of bacteriophages infecting other species in lactic acid bacteria

To our knowledge, the lysins of bacteriophages infecting the species *Streptococcus salivarius* subsp. *thermophilus* as well as those infecting pediococci have not been described. The lysin of a bacteriophage (46-8-56) infecting strains of *Leuconostoc dextranicum* was partially purified by ammonium sulphate precipitation (Shin and Sato 1980). The lytic spectrum of this enzyme on the main group of lactic acid bacteria was prepared (Table 3), and was again much wider than the phage spectrum itself.

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