Characterization of cryptic prophages (monocins) in *Listeria* and sequence analysis of a holin/endolysin gene

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Monocins in *Listeria* were induced by UV-irradiation of liquid cultures, and defective phage particles were purified from the lysates. Electron microscopy showed flexible, non-contractile bacteriophage-tail-like particles, consisting of specific proteins of molecular mass 20–45 kDa and pI 4.6–6.7. These particles were able to lyse listerial cells. DNA sequence homologies between chromosomal DNA of monicin-producing strains and labelled *Listeria* phage DNAs were inferred from DNA/DNA hybridizations, suggesting that most of the prophage DNA is still present in the listerial chromosome. An endolysin gene *cpl2438* was cloned from listerial chromosomal DNA and was identified by its expression of lytic activity against *Listeria* cells in a bioassay. The gene consists of 864 nt encoding a protein of 287 aa with a calculated molecular mass of 32 975 Da (CPL2438). This is in good agreement with the size of a protein observed in SDS-PAGE after overexpression of the lytic protein in *Escherichia coli*. The nucleotide sequence of a putative holin gene (*hol2438*, 291 nt) upstream of *cpl2438* was determined after PCR-amplification of listerial DNA and it shows typical features common to the holin gene family. Expression of the encoded protein (HOL2438, 95 aa, 10.1 kDa) in *E. coli* was found to be lethal for the host cells. The results underline the close relationship between monocins and intact *Listeria* bacteriophages, indicating that monocins are incompletely assembled phage particles derived from cryptic prophages of *Listeria*, probably including the phage lysin.

Keywords: defective phage, monicin, *Listeria*, endolysin, holin

INTRODUCTION

The ability of bacteria to produce bacteriocins or defective phages is widespread. Tagg *et al.* (1976) suggested criteria to differentiate 'true' bacteriocins of relatively low molecular mass from larger 'bacteriocin-like' substances (Hoover, 1992; Reeves 1972). Particles morphologically related to phages, such as structures resembling phage tails, occur in a variety of Gram-positive and Gram-negative bacteria. Although they do not cause productive infection (Garro & Marmur, 1970), some still possess lytic activity. Detailed analysis is severely hindered by their inability to form plaques. Defective phages display characteristics similar to both bacteriophages and bacteriocins. Tail length and number of cross-striations on extended tails have been used for differentiation and

characterization (Steensma *et al.*, 1978). Some morphologically intact phages exhibit bacteriocin-like behaviour (e.g. *Bacillus* phage ϕ BA1), and show non-productive killing activity without expression of the phage genome, which might be caused by a proteinaceous component (Ito *et al.*, 1986). This phenomenon was also noted during phage-typing of *Listeria*, where otherwise resistant strains were non-specifically inhibited by concentrated phage suspensions (Loessner & Busse, 1990).

Some of these bactericidal substances of *Listeria* strains were reported to be analogous to colicins or colicin-like agents (Sword & Pickett, 1961). Other authors reported that rod-shaped particles (rhapidosomes; Reichle & Lewin, 1967) or polysheath-tails produced during T4 infections (Moody, 1967) resemble particles isolated from *Listeria*, which have been described electron microscopically by Bradley & Dewar (1966) and Bradley (1967). Since then, many *Listeria* strains were reported to produce bactericidal substances, previously designated as mono-

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cins or listeriocins (Hamon & Péron, 1963; Ortel, 1978; Wilhelms & Sandow, 1989). Curtis & Mitchell (1992) and Mollerach *et al.* (1988) described these substances to be more similar to bacteriocins, but did not perform any electron microscopical examinations.

Although monocins are useful in *Listeria* typing (Ortel, 1978; Curtis & Mitchell, 1992; Zink *et al.*, 1994), none have yet been examined in detail. First, the aim of the present study was to investigate relationships between monocins and intact *Listeria* phages using protein data, morphological studies and DNA/DNA hybridization. Second, the molecular basis of the lytic activity of monocins was studied. Cell-wall hydrolysis by phage depends on the synthesis of endolysins (Foster, 1993; Longchamp *et al.*, 1994; Shearman *et al.*, 1994) and, in many cases, of holins forming pores in the cytoplasmic membrane (Arendt *et al.*, 1994; Henrich *et al.*, 1995; Steiner *et al.*, 1993; Young, 1992). We show that a monocin-carrying *Listeria* strain in fact harbours a holin-endolysin system.

METHODS

Induction of defective phages and activity testing of monocins. A set of 144 *Listeria* strains was initially screened for listeriocidal activity after induction with UV light (254 nm, 0.0132 J cm⁻²), using the soft-agar-layer method, where defective phages do not form plaques. Seven monocin producers were then selected for further studies (Table 1). Tryptose broth and agar (1.2%, Merck) and an incubation temperature of 30 °C were used for *Listeria* throughout. *Listeria* indicator strains from different species and serovars are given in Table 1. Thermal inactivation of monocins was determined by incubation (2 ml samples) at different temperatures (30, 40, 45, 50, 60, 70 and 80 °C) and variable times (1, 2.5, 5, 10, 15 and 30 min). For determination of activity at different pH values, dilutions of monocins were incubated for 2 h in SM-buffer (see

below), adjusted to pH 2–12. Thereafter, monocins were diluted 100-fold in SM-buffer (pH 7.5) and the remaining activity was determined in comparison with untreated samples as described previously (Zink *et al.*, 1994). In short, determination of lytic ranges was done by applying the spot-on-the-lawn technique and 24 h incubation. Activity was then recorded in three grades: confluent lysis, turbid zone and no lysis. The lytic range of the monocins was established using the following 41 strains of 22 genera of Gram-positive bacteria from our culture collection: *Arthrobacter* (2 strains); *Bacillus* (10); *Brevibacterium* (2); *Brochothrix* (1); *Cellulomonas* (1); *Corynebacterium* (2); *Curtobacterium* (1); *Enterococcus* (2); *Erysipelothrix* (1); *Jonesia* (1); *Kurtzia* (1); *Lactobacillus* (4); *Lactococcus* (2); *Leuconostoc* (1); *Microbacterium* (1); *Micrococcus* (2); *Nocardia* (1); *Oerskovia* (1); *Pediococcus* (1); *Propionibacterium* (1); *Rhodococcus* (1); and *Staphylococcus* (2).

Concentration and purification. Two litres of a UV-induced culture (6 h incubation at 30 °C) were centrifuged at 22000 g (20 min, 10 °C). Bacterial cells were removed from the supernatant by a passage through a 0.22 µm pore-size filter and subsequently concentrated by ultrafiltration to a final volume of 150 ml (molecular mass cut-off > 20 kDa; Sartocoon Mini Cross-flow system, Ultrasart module, Sartorius). Monocins were precipitated overnight at 4 °C with 10% (w/v) PEG-8000 (Yamamoto *et al.*, 1970), collected by centrifugation (15000 g, 20 min, 4 °C), and resuspended in 3 ml SM-buffer (Sambrook *et al.*, 1989). Chloroform-extraction eliminated residual PEG-8000. Particles were then purified on a pre-formed stepped CsCl-gradient (density 1.1–1.5 g cm⁻³) at 93000 g for 15 h at 4 °C. Opalescent bands at a buoyant density of approximately 1.3 g cm⁻³ were collected, washed in SM-buffer (216000 g, 90 min, 4 °C), and resuspended in Milli-Q water (Millipore).

Electrophoretic analyses of proteins and electron microscopy. These were performed as described by Zink & Loessner, (1992) and Loessner *et al.* (1994b).

Extraction and purification of *Listeria* genomic DNA. Cells were lysed as described by Loessner *et al.* (1995a). Samples were deproteinized by extractions with phenol and chloroform. The

Table 1. Monocin producers and indicator strains of *Listeria*

Species	Serovar	WSLC code	Origin, synonym†
Monocin producers			
<i>L. monocytogenes</i>	'7'	1034	SLCC 2482
<i>L. monocytogenes</i>	1/2a	1040*	SLCC 53/ATCC 15313
<i>L. monocytogenes</i>	1/2a	1132	Food
<i>L. innocua</i>	6a	2011*	SLCC 3379/ATCC 33090
<i>L. innocua</i>	6b	2438	Food
<i>L. ivanovii</i>	5	3010*	SLCC 2379/ATCC 19119
<i>L. welshimeri</i>	6b	5008*	SLCC 5334
Indicator strain			
<i>L. monocytogenes</i>	4b	1003	SLCC 2375/ATCC 19115
<i>L. monocytogenes</i>	4c	1019	SLCC 2376/ATCC 19116
<i>L. monocytogenes</i>	'7'	1034	SLCC 2482
<i>L. monocytogenes</i>	4b	1042	ATCC 23074
<i>L. innocua</i>	6b	2023	SLCC 5640
<i>L. ivanovii</i>	5	3009	SLCC 4769

* Type strains.

† ATCC, American Type Culture Collection, Rockville, MD, USA; SLCC, Special *Listeria* Culture Collection, Würzburg, Germany; WSLC, Weihenstephan *Listeria* Collection, Freising, Germany.

DNA was then ethanol precipitated, resuspended in TE-buffer (Sambrook *et al.*, 1989), and stored at 4 °C. Concentrations were determined photometrically.

DNA/DNA hybridizations. Purified DNA from monicin-producing strains was spotted (0.25 µg DNA per spot) onto positively charged nylon membranes (Boehringer), and covalently linked to the membranes by UV irradiation (0.7 J cm⁻², 312 nm). DNA from 19 different *Listeria* phages of various morphological types and species (A511, A005, A006, A500, A502, A620, A640, B021, B025, B035, B054, B056, B101, D441, 10072, 13441, 90666, 90861, PSA; Zink & Loessner, 1992; Loessner *et al.*, 1994a, b) were digoxigenin-labelled (Random-Primed DNA Labelling kit, Boehringer), and hybridized to the membrane-bound *Listeria* chromosomal DNA. Hybridization and signal detection were performed under high-stringency conditions by chemiluminescence as described previously (Loessner *et al.*, 1994b). DNA from selected monicin-producers displaying strong dot-blot signals (*Listeria* WSLC1040/phage A005, WSLC1132/A005, WSLC2438/A500 and WSLC2438/A620), were then *Cla*I-digested and Southern-hybridized to the respective phage DNAs.

Cloning and sequencing of the endolysin gene. For expression-library construction, DNA of the monicin producer WSLC2438 was partially digested with *Taq*I (Promega) and separated by agarose gel electrophoresis. Fragments between 1.5 and 3.5 kb were excised from 0.8% low-melting-point agarose gels (SeaPlaque GTG, FMC Bioproducts). After β -Agarase (USB) digestion and concentration by ultrafiltration (Microcon-100, Amicon), DNA-fragments were inserted (T4 DNA ligase, Boehringer) into plasmid pSP72 (Promega), which had been linearized with *Cla*I (Boehringer). Ligation products were de-salted and transformed by electroporation into *E. coli* JM109(DE3). For preparation of competent cells, see Dower *et al.* (1988). To detect lysin activity, replicas from the original plates were created on plates containing 0.4 mM IPTG. After 5 h, cells were exposed to saturated chloroform vapour for 5 min, overlaid with a 5% inoculum of a 100-fold-concentrated culture of *Listeria* in 0.4% agar, and incubated at room temperature for 1–3 h.

The nucleotide sequence of the DNA fragment present on pCPL2438 was determined by the dideoxy chain-termination method according to the manufacturer's instructions (Sequenase version 2.0, USB), using a primer-walking strategy.

Cloning of the holin gene. The holin gene was amplified by PCR from chromosomal DNA of WSLC2438. A holin downstream primer (5'-ACATGAATTCATGATGAAATGGA-GTTTGGAAAAGAGTTACT-3') was deduced from the short nucleotide sequence upstream of the lysin gene *cp12438*. The holin upstream primer (5'-ATCAGAATTCCTTATTA-ATCATCCTTCCATATTTTGTAGCTC-3') was inferred from a closely related holin gene (*hol500*) of *Listeria* phage A500 (Loessner *et al.*, 1995b). Both primers included a 5' *Eco*RI site (underlined). PCR was performed with *Taq* polymerase according to the manufacturer's instructions (PCR-Core kit, Boehringer). PCR products were extracted with phenol and chloroform, ethanol precipitated, and resuspended in 0.1 × TE-buffer (pH 8.0). After *Eco*RI digestion, fragments were ligated into pBluescriptII (Stratagene) and transformed into *E. coli* DH5 α (Life Sciences). Screening for *hol2438* recombinant clones was done by α -complementation on IPTG- and X-Gal-supplemented agar plates, and by restriction enzyme analysis of isolated plasmids. Nucleotide sequencing of the insert was performed as described above.

DNA sequences and proteins translated from the putative ORFs

were analysed by the DNASIS/PROSIS program package (Hitachi). We used GenBank (R81.0) and EMBL (R38.0) databases for DNA analyses, and NBRF-PIR (R40.0) and Swiss-Prot (R28.0) for protein analyses, respectively.

RESULTS

Monocins resemble phage tails and specifically kill *Listeria*

Electron microscopy revealed that all monocins investigated are similar to bacteriophage tails (Fig. 1a) and resembled flexible, non-contractile tails, which are uniform in size (about 110–120 nm long and 11 nm wide) and have 25–26 striations. Occasionally, a few assemblies of phage tails attached to apparently empty heads and various rod-shaped structures resembling long tubes were observed (Fig. 1b). Electron microscopic observation of cell lysis after incubation of sensitive cells with monocins M1040 and M2438 demonstrated that the total cell surface was covered with adsorbed particles (Fig. 1c). Ruptures and cytoplasmic efflux were obvious and monocins were attached only by their receptor structure to the cell wall (Fig. 1d). Adsorption was strain-specific, and none of the other Gram-positive genera tested were sensitive to monocins.

Protein composition and stability of monocins

The particles exhibited protein patterns with major bands ranging from 30 to 31 kDa and some weak minor proteins (Fig. 2a). Monocin M1040 was somewhat different with proteins of approximately 20, 21, 23, 29 and 45 kDa. Protein patterns obtained by isoelectric focusing are depicted in Fig. 2b. All monocins investigated showed one (or more) dense protein bands and a number of weak bands. Most proteins had pIs between pH 4.9 and 5.1. M1040 revealed proteins in the pI range of pH 4.6–6.7. Monocin activity was found to be stable between 30 °C and 40 °C, and pH 3–11. During 12 min at 45 °C, activity was reduced by approximately 50%. However, 50 °C completely inactivated lytic activity. Storage at 4 °C for some weeks had little or no effect on monocins, and monocins were stable during storage at –20 °C.

Homology with DNA of intact *Listeria* phages

Fourteen of 19 *Listeria* phage DNAs hybridized to the DNA of one or more monicin-producing *Listeria* strains. None of the Myoviridae phages (morphotype A1: A511, B054; Zink & Loessner, 1992) showed any homology, neither did 3 of the 17 Siphoviridae (morphotype B1) tested. Strong hybridization signals were observed between monicin-producing *Listeria* strains WSLC1034, WSLC1132, WSLC2438 and phages A500, A006, B025, A620 and A005 (Fig. 3a). *Listeria* strains WSLC1040, WSLC2011 and WSLC3010 showed weak or no signals. Southern blots with *Cla*I-digested DNA (Fig. 3b) enabled us to summarize the size of all labelled fragments, resulting in 35–40 kb, which reflects the genome size of intact Siphoviridae phages (Loessner *et al.*, 1994b).

Fig. 1. (a) Monocin M1040; bar, 100 nm. (b) Rod-shaped structures observed in some monocin preparations (M2011); bar, 100 nm. (c) *Listeria* cell (WSLC 3009) lysed by monocin M1040 after 30 min incubation: cell wall is severely destructed; bar, 500 nm. (d) Enlarged section of the cell surface with monocin particles attached; bar, 100 nm. All specimens were negatively stained with 2% uranylacetate.

Monocin-producing *Listeria* WSLC2438 harbours a lysin gene (*cpl2438*)

Expression and screening of clones containing DNA fragments of strain WSLC2438 led to the identification of a clone with lytic activity against *Listeria* cells. When induced with IPTG, colonies showed a clear inhibition zone in a lawn of *Listeria* cells in an overlay test on agar plates after 30 min (Fig. 4a). Restriction analysis of the isolated plasmid showed the presence of a 2.1 kb DNA insert. The nucleotide sequence was determined and revealed an 864 nt ORF, encoding a protein of 287 aa with a calculated molecular mass of 32975 Da (Fig. 5). The start codon was preceded by a possible RBS. A 20 kb band from *Cla*I-digested DNA fragments from monocin-producing *Listeria* hybridized with digoxigenin-labelled pCPL2438, carrying the 0.9 kb fragment of *cpl2438* (Fig. 3b). Extensive hybridizations with 22 monocin-producing strains revealed that only six strains (WSLC1034, WSLC1132, WSLC2105, WSLC2438, WSLC3010, WSLC5008) exhibited positive signals.

A putative holin gene (*hol2438*) is located upstream of *cpl2438*

Upstream of the endolysin gene, the 2.1 kb fragment contains 34 nt which were similar to a holin sequence known from a *Listeria* phage (Loessner *et al.*, 1995b).

After PCR-amplification from chromosomal DNA (for PCR primers see Methods), a major band with a size of about 300 bp was found. Subcloning and sequencing revealed an ORF for a putative holin gene (*hol2438*, 291 nt, Fig. 5). The gene product HOL2438 consists of 95 aa and has a calculated molecular mass of 10.1 kDa with a structure similar to other holin proteins (for details see discussion).

Overexpression and lytic range of CPL2438

E. coli JM109(DE3)(pCPL2438) cells were grown to OD₆₀₀ 0.5 before induction with 0.4 mM IPTG, followed by further incubation and sampling at different times. Total protein content of the cells was determined with SDS-PAGE. Starting at 30 min after induction, we observed a 33 kDa protein (Fig. 4b). The molecular mass is in agreement with that of the predicted phage endolysin. Production of CPL2438 increased steadily, reaching a maximum at 5 h. Clones without addition of IPTG showed no expression. Lytic activity studies with more than 70 different *Listeria* strains (all species and serovars, detailed data not shown) revealed a rapid decrease in OD₆₀₀ for all tested *Listeria* cells, indicating cell destruction. Apart from three *Bacillus* spp. (*B. megaterium*, *B. polymyxa*, *B. subtilis*), none of the other 38 Gram-positive strains tested (see Methods) showed sensitivity to CPL2438.

Fig. 2. Electrophoretic analyses of monocin proteins. Lanes: 1, M1040; 2, M1034; 3, M1132; 4, M2011; 5, M2438; 6, M3010; 7, M5008. (a) SDS-PAGE protein profiles. Molecular masses of the marker proteins (SDS-7L, Sigma) are indicated on the left. (b) Isoelectric focusing in immobilized pH gradients. The linear pH gradient is indicated on the right.

DISCUSSION

Bactericidal substances are produced by *L. monocytogenes* (Curtis & Mitchell, 1992; Hamon & Péron, 1963; Mollerach *et al.*, 1988; Ortel, 1978; Wilhelms & Sandow, 1989), but were not studied in structural detail. Sword & Pickett (1961) reported that their *Listeria* strains produced substances 'possibly analogous to colicins or colicin-like agents'. Using electron microscopy, we observed structures which are similar to non-contractile, flexible phage tails (Fig. 1). Bradley & Dewar (1966) described longer particles (240 *vs* 120 nm), which resemble contractile phage tails. In no case did we observe monocins resembling contractile phage tails. All monocins differ from structures described for *E. coli* (Medoff & Swartz, 1969), *Pseudomonas* spp. (R-type 'Pyocins'; Ishii *et al.*, 1965; Higerd *et al.*, 1969; Shinomiya & Shiga, 1979) or *Bacillus* (Steensma & Robertson, 1978; Steensma *et al.*, 1978; Wood *et al.*, 1990). Protein profiles showed that six of the seven monocins investigated (except M1040) exhibited patterns relatively similar and therefore would

Fig. 3. (a) Dot-blot hybridization of membrane-bound DNA of six monocin-producing *Listeria* strains (WSLC 1034, 1040, 1132, 2011, 2438 and 3010) to digoxigenin-labelled listeriophage DNA (A511, A500, A006, B025, PSA, A620, A005, 90861); (b) Southern-blot hybridization of *Clal*-digested chromosomal DNA fragments from monocin-carrying *Listeria* strains WSLC 1040, 1132 and 2438. Fragments were separated by electrophoresis (0.8% agarose gels) and hybridized to digoxigenin-labelled listeriophage DNA. Lanes: λ , molecular mass markers; 1, WSLC1040/phage A005; 2, WSLC 1132/phage A005; 3, WSLC 2438/phage A500; 4, WSLC 2438/phage A620. Arrow indicates endolysin-encoding fragment of WSLC 2438, identified with digoxigenin-labelled probe pCPL2438

seem to be related to each other (Fig. 2). When compared to *Listeria* phage-protein profiles (Loessner *et al.*, 1994a, b; Zink & Loessner, 1992), monocin proteins correspond in molecular mass and pI with major proteins of phage

Fig. 4. (a) Expression of the endolysin gene *cpl2438* from plasmid pCPL2438 in *E. coli* JM109(DE3). Cells were chloroform-treated and overlaid with a lawn of *Listeria* cells. Distinctive zones of lysis are visible around the colonies; (b) Overexpression of CPL2438 (arrow) in *E. coli* JM109(DE3) carrying pCPL2438. Induction was done by adding 0.4 mM IPTG to exponential-phase cells. Samples were collected at indicated time-points. Electrophoresis patterns of total cell proteins were determined by SDS-PAGE.

tails. It was, therefore, not surprising that 75% of the *Listeria* phage DNAs hybridized to DNA of monocin-producing listeriae.

Based on these data, we conclude that monocins are inducible defective prophages, which are present, if at all, in very low numbers in non-induced bacterial cultures. Since most of the genetic information for a Siphovirus (35–40 kb) may still be present in monocin-carrying *Listeria* (Fig. 3b), we suggest that errors in phage morphogenesis rather than extensive deletions are leading to incompletely assembled phages.

Our observation that none of the monocins inhibited any other Gram-positive bacteria is in contrast to earlier reports stating that monocins display a much broader activity spectrum and are active against 30% of *Staphy-*

Fig. 5. Nucleotide sequence and inferred amino acid sequence of the *cpl2438* (nt 291–1154) and putative *hol2438* gene (1/10–291). The DNA sequence of nt 1–257 was determined after PCR-amplification. PCR primers are indicated by dashed lines. A potential RBS (nt 275–281) for *cpl* is underlined; start codons are in raised letters and stop codons are in lowered letters and are designated with asterisks.

lococcus spp. and *Bacillus* spp. tested (Hamon & Péron, 1963; Ortel, 1978). Regarding the strictly genus-specific action of *Listeria* phages (Loessner & Busse, 1990; Loessner, 1991), transgeneric inhibition is most probably not due to defective phages. This would also explain the contradiction between our finding that only a relatively small fraction of *Listeria* strains (17%) produce true defective phages and reports of other groups, which stated that 58% (Wilhelms & Sandow, 1989), 76% (Ortel, 1978) or 98% (Curtis & Mitchell, 1992) of *Listeria* strains produce bactericidal substances. Following Bradley & Dewar (1966), and in order to clarify the nomenclature, we suggest that the term 'monocin' should be used only when the bactericidal principle is a defective phage particle, as confirmed by electron microscopy. In all other cases, the term 'bacteriocin' or 'listeriocin' seems to be more adequate (Curtis & Mitchell, 1992; Mollerach *et al.*, 1988).

From previous studies with defective phages of *B. subtilis* (Steensma, 1981), it was concluded that the target of lytic activity could be either the cell wall or the cell membrane. Adsorption occurs at a cell-wall receptor and isolated defective phages were shown to kill without irreversible adsorption. In other defective prophages (Foster, 1993) and bacteriophages (Young, 1992), lytic enzymes have been identified as the killing principle. For the defective *Bacillus* phage PBSX, Longchamp *et al.* (1994) clearly identified host lysis genes inserted between structural and regulative genes.

Until now, no information about the molecular killing mechanism of monocins was available. We observed monocin-induced lysis of listerial cells (Fig. 1d), which indicates the presence of a lytic enzyme in monocins. A 2.1 kb fragment isolated from an expression library clearly showed a lytic phenotype which is due to the synthesis of a 33 kDa protein (Fig. 4), most probably an endolysin, corresponding to an 864 bp ORF, *cpl2438* (Fig. 5). No homologous proteins were found in protein database searches. Labelled *cpl2438* did not hybridize to DNA from all monocin-producing strains, suggesting different lysin gene structures. No ORF could be identified downstream of *cpl2438*, although hybridization to *Listeria* phage-DNA was found. Our data, however, do not suggest regulative functions for these sequences.

The endolysin gene *cpl2438* is preceded by a small ORF, designated *hol2438*, encoding a 95 aa hydrophobic protein. Although no similarity to other proteins was found in database searches, motifs such as transmembrane segments and spatial distribution of α -helices and β -turns (data not shown) were clearly similar to holins described for other bacteriophages (for review see Young, 1992), especially for *Listeria* phages (Loessner *et al.*, 1995b). This similarity includes a dual start motif, permitting synthesis of two different proteins enabling the regulation of the pore-forming process (for details see lambdoid S gene in Young, 1992), and an overlap of the putative stop codon of *hol2438* and the putative start codon of *cpl2438* (Fig. 5). Holins were shown to form pores in the cytoplasmic membrane, providing access for the endolysin to the cell wall of the host cell (Young, 1992). Clearly, the expression of holins is expected to be lethal. From sequence analysis of nine clones of the PCR amplified putative holin gene we have found that subcloning of this gene was only possible when it was inserted in a 3' $\rightarrow$ 5' direction distally from the T7 promoter of pSP72, leading only to transcription of the non-coding strand and, thus, effectively preventing expression (cf. Steiner *et al.*, 1993; Young, 1992).

Other results of our laboratory clearly show a similar organization of the cloned lysis cassettes (holin and endolysin) of defective and intact phages of *Listeria* (Loessner *et al.*, 1995b). The temperate siphoviral phage A500 harbours the lysis gene *ply500* (867 nt) which encodes a phage lysin of 289 aa with 33.4 kDa. Overall DNA sequence identity between *cpl2438* and *ply500* is 86 %, and 95 % between *hol2438* and the A500 holin gene. From our data we conclude that the monocin-producing

L. innocua WSLC2438, among other genes of a defective prophage, harbours a functional phage holin-endolysin system derived from a *Listeria* phage. Certainly, this system is used for cell lysis when monocins are produced (lysis from within). Monocin-induced lysis of sensitive listeriae (lysis from without) is coupled to an adsorption of monocins to the cell wall (Fig. 1d). However, whether the cloned lysin, perhaps as part of the baseplate or receptor structure of the defective phage, indeed contributes to the lytic mechanism of monocins, is not yet proven.

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