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Molecular Characterization of a New Abortive Infection System (AbiU) from *Lactococcus lactis* LL51-1

GANG DAI,[1] PING SU,[1,2]* GWEN E. ALLISON,[2] BRUCE L. GELLER,[3] PING ZHU,[2] WOOJIN S. KIM,[1] AND NOEL W. DUNN[1]

Department of Biotechnology, The University of New South Wales, Sydney, New South Wales 2052,[1] and DSM Food Specialties, Moorebank, New South Wales 1875,[2] Australia, and Department of Microbiology, Oregon State University, Corvallis, Oregon 97331-3804[3]

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This study reports on the identification and characterization of a novel abortive infection system, AbiU, from *Lactococcus lactis*. AbiU confers resistance to phages from the three main industrially relevant lactococcal phage species: c2, 936, and P335. The presence of AbiU reduced the efficiency of plaquing against specific phage from each species as follows: 3.7×10^{-1}, 1.0×10^{-2}, and 1.0×10^{-1}, respectively. *abiU* involves two open reading frames, *abiU1* (1,772 bp) and *abiU2* (1,019 bp). Evidence indicates that AbiU1 is responsible for phage resistance and that AbiU2 may downregulate phage resistance against 936 and P335 type phages but not c2 type phage. AbiU appeared to delay transcription of both phage 712 and c2, with the effect being more marked on phage c2.

Lactococcus lactis is the main bacterial species used as starter cultures in dairy fermentations. However, phage infection of this species is a problem that causes significant economic loss. Research on natural phage resistance systems encoded by *Lactococcus* led to the identification of four different mechanisms: adsorption inhibition, DNA injection blocking, restriction-modification (R/M), and abortive infection (abi) (for a recent review, see reference 12). The first three systems operate at steps early in the phage infection process, whereas abortive infection can target various different phases of phage development, including DNA replication, RNA transcription, protein synthesis, packaging, and morphogenesis.

To date, 18 abortive infection systems have been sequenced and are designated AbiA through AbiR (7, 12). DNA and protein sequence analysis indicates that none of the abi systems has any significant homology with other genes and proteins in the databanks. In addition, few motifs have been identified, making it difficult to hypothesize the function(s) of each protein (12). While comparison of the different abi systems revealed that AbiD, AbiD1, and AbiK proteins are related, they have been reported to have very different modes of action (1, 11, 22). Fourteen of the eighteen abi systems sequenced are encoded by single genes. Four abi systems—AbiE, AbiG, AbiL, and AbiR—involve two genes (6, 7, 13, 26). DNA sequence analysis, Northern hybridization, and reverse transcription-PCR demonstrated that the two genes of AbiE, AbiG, and AbiL are cotranscribed (6, 13, 26). AbiR is encoded by two separate genetic loci (7).

The mode of action of various different abi systems have been studied. AbiA, AbiF, and AbiK were found to interfere with phage DNA replication (11, 13, 19). AbiQ was found to cause accumulation of the replicative form of phage DNA so that it could not be cleaved into the mature form. AbiB and AbiG were demonstrated to interfere with phage RNA transcription (26, 27). AbiD1 has been shown to interact with a phage operon consisting of four genes (1). AbiD1 appears to act in cooperation with Orf1 to decrease the level of Orf3 to below that required for proper phage development (1). In the presence of AbiA, the appearance of major capsid protein (MCP) of phage ul36 was delayed compared to that produced in the sensitive host (24). AbiC reduces the amount of MCP production (9). Like AbiA, AbiK also inhibited phage ul36 DNA replication. As a consequence, MCP of phage ul36 could not be detected in $AbiK^+$ cells. It is believed that AbiK acts prior to phage DNA replication (11). MCP was produced normally in $AbiQ^+$ cells, but the lytic cycle of the phage was blocked after MCP synthesis (12). The mechanistic studies clearly indicate that the different abi systems act on different phage targets at various stages of development.

The various abi systems affect the three main species of lactococcal phages (c2, 936, and P335) differently. Some Abis, such as AbiB, AbiE, AbiH, and AbiJ, are effective against only one of the three main species of lactococcal phages. Others, such as AbiC, AbiD, AbiD1, AbiF, AbiG, AbiI, and AbiL, inhibit propagation of two species of phage. For AbiA and AbiK, representatives of all three phage species are inhibited.

This study describes the isolation and molecular characterization of a novel abortive infection phage resistance system, AbiU, from a native plasmid that was isolated from a phage-resistant industrial strain, *L. lactis* LL51-1.

MATERIALS AND METHODS

Bacterial strains, plasmids, phages, and media. The strains and plasmids used in this study are listed in Table 1. *Escherichia coli* was grown at 37°C in Luria-Bertani medium (30). *L. lactis* was grown at 30°C in M17 medium (31) supplemented with 0.5% glucose (M17G). For propagation of phages, calcium chloride (10 mM) was added to M17G medium. For selection and plasmid maintenance, antibiotics were added to the medium as follows: for *E. coli*, 100 μg of spectinomycin per ml; for *L. lactis*, 500 μg of spectinomycin, 300 IU of nisin, and 500 μg of streptomycin per ml.

* Corresponding author. Mailing address: Department of Biotechnology, University of New South Wales, Sydney, NSW 2052, Australia. Phone: 61-2-93853868. Fax: 61-2-93136710. E-mail: p.su@unsw.edu.au.

TABLE 1. Bacterial strains, plasmids, and phages

Strain, plasmid, or phage	Relevant characteristics[a]	Source or reference
Strain		
L. lactis subsp. *lactis*		
LL51-1	Industrial strain, *lac*$^+$ *prt*$^+$ Nis^r	DSM Food Specialties
LM0230	*lac prt* plasmid-free derivative of c2	10
LM0230Sm^r	Sm^r derivative of LM0230	8
L. lactis subsp. *cremoris* UL8		25
E. coli NM522	Transformation host	17
Plasmids		
pDL278	Spec^r *E. coli-L. lactis* shuttle vector (6.6 kb)	21
pND001	Native plasmid of LL51-1, 75 kb	This study
pND002	5.2-kb *Hin*dIII fragment from pND001 cloned into pDL278	This study
pND003	*Bam*HI deletion of pND002 (*abiU1* deletion)	This study
pND006	*abiU2* deletion of pND002	This study
Phage		
ϕ712	Small isometric-headed phage, 936 species propagated on LM0230	14
ϕc2	Prolate-headed phage, c2 species propagated on LM0230	12
ϕul36	Small isometric-headed phage, P335 species propagated on UL8	25

[a] Spec^r, Nis^r, and Sm^r, spectinomycin, nisin, and streptomycin resistance, respectively.

Conjugation and transformation. Conjugation was carried out by filter mating (15). *L. lactis* was transformed by electroporation as described by Powell et al. (28). For *E. coli*, the $CaCl_2$ transformation method was used as described by Sambrook et al. (30).

Phage techniques. Cross-streaking was employed for the initial screening of phage-resistant and phage-sensitive isolates. Phage preparations were titered by a standard plaque assay (20). The efficiency of plaquing (EOP) was calculated by dividing the phage titer on test strains by that on the sensitive host LM0230. The efficiency of center of infection (ECOI) assays were conducted as described by Deng et al. (6). By convention, it is assumed that 100% of the infected sensitive cells result in progeny phage (ECOI = 1). Cell survival was determined as described by Behnke and Malke (3). The cell survival rate was calculated as the colony count of infected culture divided by colony count of uninfected control. One-step growth curves were carried out by the method of Klaenhammer and Sanozky (20).

Plasmid DNA techniques. Lactococcal plasmid DNA was isolated by the method of Anderson and McKay (2). Plasmid was isolated from *E. coli* by alkaline lysis and purified by cesium chloride-ethidium bromide density gradient centrifugation (30). Molecular cloning was essentially carried out as described by Sambrook at al. (30). Restriction endonucleases and T4 DNA ligase were purchased from Roche Molecular Biochemicals (Roche Diagnostics Australia Pty., Ltd.) or New England BioLabs (Genesearch, Pty., Ltd.) and used as recommended by the manufacturers.

DNA cloning. Restriction endonuclease, calf intestinal alkaline phosphatase, and T4 DNA ligase were purchased from Roche and used in cloning work according to the manufacturer's instructions. DNA cloning procedures were as described by Sambrook et al. (30). Plasmid pND002 was obtained by shotgun cloning a *Hin*dIII digest of pND001 into the *Hin*dIII site of pDL278.

Inactivating *abiU1* was achieved by digesting pND002 with *Bam*HI, gel purifying the largest fragment, and religation to produce pND003 (see Fig. 3). Plasmid pND002 was double digested with *Hin*dIII and *Sca*I, and the 2.9-kb fragment was gel purified and cloned into the *Hin*dIII/*Sca*I sites of pDL278 to create pND006 (see Fig. 3). The constructs were confirmed by restriction enzyme mapping.

Nucleotide sequencing and analysis. Both DNA strands were sequenced by using an Applied Biosystems 377 DNA sequencer according to the manufacturer's protocol. Sequencing of the phage resistance determinant was initiated by using the M13mp19 primers (New England Biolabs). Based on the sequences obtained, 17- to 21-mer oligonucleotide primers were synthesized and used to "walk" along the DNA template. Recording and analysis of the nucleotide sequence were carried out by using AutoAssembler DNA sequence assembly software (Applied Biosystems) and the Australian National Genomic Information Service (ANGIS) Software System operated by ANGIS at the University of Sydney.

Northern blot hybridization. Total RNA was isolated from *L. lactis* at various times during the phage infection cycle by using the RNeasy Mini Kit (Qiagen, Pty., Ltd.) according to the manufacturer's instructions. RNA preparations were digested with RQ1 DNase (Promega Corp., Sydney, Australia) prior to use. RNA concentrations were determined spectrophotometrically at 260 nm. Northern hybridization was carried out by using the ECL Direct Nucleic Acid Labeling and Detection System (Amersham Pharmacia Biotech) according to the manufacturer's instructions. Total RNA (12 μg) from each sample was loaded onto a 1.2% denaturing gel. Phage DNA probes were isolated by the method of Grosserberger (18) and purified by using the QIAEX II gel extraction kit (Qiagen).

Nucleotide sequence accession number. The GenBank accession number for the DNA sequence of the AbiU abortive infection system from *L. Lactis* LL51-1 is AF188839.

RESULTS

Isolation and cloning of the phage resistance determinant. *L. lactis* LL51-1 is an industrial cheese starter strain that shows good phage resistance (ϕ^r). Characterization of this strain also revealed that it contains nine plasmids (of ca. 75, 60, 50, 45, 27, 10, 9, 8, and 3 kb) and is sensitive to streptomysin (Sm^s) and resistant to nisin (Nis^r).

In an attempt to identify the plasmid encoding phage resistance, conjugation experiments were conducted between LL51-1 (Nis^r ϕ^r Sm^s) and LM0230Sm^r (Nis^s ϕ^s Sm^r), and transconjugants were obtained at 10^{-7} per donor on M17G plates containing streptomycin and nisin. Nine transconjugants were resistant to phages ϕc2 and ϕ712, which are representatives of the c2 and 936 lactococcal phage species, respectively. Two of the nine transconjugants contained a single plasmid of ca. 75 kb, and *Hin*dIII digests of plasmid DNA from both transconjugants produced identical restriction patterns. This 75-kb plasmid was designated pND001. When LM0230 (pND001) was infected separately with ϕc2 and ϕ712, reductions in EOP were observed (3×10^{-1} and 2×10^{-4}, respectively), and plaque sizes were reduced relative to LM0230 (Table 2).

To isolate the region of pND001 responsible for phage resistance, *Hin*dIII restriction fragments of pND001 were shot-

TABLE 2. Phage reaction on *L. lactis* LM0230(pDL278), LM0230(pND002), UL8(pDL278), UL8(pND002), and derivative plasmids at 30°C

Strain	Phage	EOP	Plaque size (mm), morphology	Adsorption (%)	Cell survival (%)
LM0230	ϕ712	1	1.5, clear	99.3	
	ϕc2	1	2.5, clear		
LM0230(pDL278)	ϕ712	1	1.5, clear	99.4	0.3
	ϕc2	1	2.5, clear		17
LM0230(pND001)	ϕ712	2.0×10^{-4}	0.2, clear	99.4	
	ϕc2	3.0×10^{-1}	1, clear		
LM0230(pND002)	ϕ712	1.0×10^{-2}	0.3, hazy	99.5	1.5
	ϕc2	3.7×10^{-1}	1, hazy		14
LM0230(pND003)	ϕ712	1	1.5, clear		
	ϕc2	1	2.5, clear		
LM0230(pND006)	ϕ712	1.0×10^{-4}	0.3, hazy		
	ϕc2	3.0×10^{-1}	1, hazy		
UL8(pDL278)	ϕul36	1	1.5, clear		5.1
UL8(pND002)	ϕul36	1.0×10^{-1}	1.5, clear		5.3
UL8(pND006)	ϕul36	4.0×10^{-2}	1.2, clear		5.7

gun cloned into pDL278 and introduced into LM0230. Recombinant clones were tested for resistance to both phages, and the plasmid content of resistant clones was analyzed. The smallest fragment from pND001 that conferred resistance to both phages was 5.2 kb. This construct was designated pND002.

Characterization of the phage resistance mechanism in pND002. Various assays were conducted to characterize the phage resistance mechanism encoded by pND002. To investigate whether the phage resistance phenotype encoded by pND002 was due to the inability of phage to adsorb to the host cells, adsorption inhibition assays were carried out by using ϕ712. The results are listed in Table 2. Phage ϕ712 adsorbed efficiently to the sensitive control strain LM0230(pDL278) and to the resistant strains LM0230(pND001) and LM0230 (pND002), indicating that the phage resistance encoded by pND001 and pND002 did not affect ϕ712 adsorption.

To investigate the effect of pND002 on phage infection, one-step phage growth curves of ϕ712 in LM0230(pDL278) and LM0230(pND002) were conducted at 30°C (Fig. 1). Compared to the sensitive control strain, LM0230(pDL278), the latent period for LM0230(pND002) was extended by 5 min (30 versus 25 min). More importantly, the burst size obtained with LM0230(pND002) was significantly reduced relative to the control. Furthermore, the size of the plaques obtained on LM0230(pND002) were much smaller than those obtained on LM0230(pDL278) (0.3 versus 1.5 mm; Table 2). These results indicate that pND002 may encode an abortive infection system.

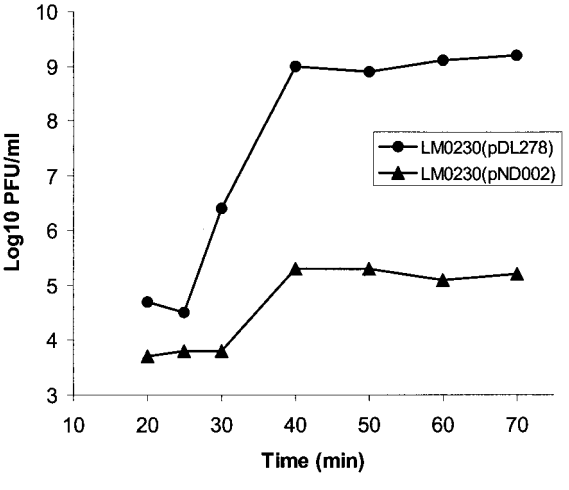

FIG. 1. One-step growth curve of ϕ712 in LM0230(pDL278) and LM0230(pND002).

Another characteristic of abortive infection is death of the host cells after phage infection. The number of cells that died as a result of ϕ712 infection was examined (Table 2). Compared to the sensitive host, the presence of pND002 in LM0230 did not improve the cell survival rate after ϕ712 infection. The LM0230(pND002) culture, however, did not lyse, whereas the LM0230(pDL278) culture did. Similar results were observed after ϕc2 infection (Table 2).

ECOI assays with ϕ712 were performed on both sensitive LM0230(pDL278) and resistant LM0230(pND002). It was shown that 3.2×10^8 infectious centers were formed on LM0230(pDL278), while only 5.0×10^6 infectious centers were formed on LM0230(pND002) under the same conditions. Therefore, the ECOI of LM0230(pND002) was 1.6%. This extremely low efficiency of infectious center formation is another typical characteristic of abortive infection systems.

Sequence analysis of the 5.2-kb insert in pND002. Sequence data showed that the exact length of the insert in pND002 was 5,213 bp. Use of the GCG programs Frame and Mapping, available through ANGIS, predicted the presence of three complete open reading frames (ORFs): *orf1*, *orf2* (designated *abiU1* and *abiU2* after characterization), and *orf3* and one truncated ORF, *orf4* (Fig. 3). The sequence of all ORFs, together with the flanking control regions, is given in Fig. 2. All three complete ORFs are read in the same direction, while *orf4* is in the opposite direction. *abiU1* is 1,766 bp long, starting at bp 1078 with AUG as the start codon and ending at bp 2844, followed by two stop codons, UAG and UAA. It has a G+C content of 26% and encodes a predicted 589-amino-acid protein of 67.9 kDa. *abiU1* is preceded by a putative promoter (−35 box [TTGATT], 16-bp spacer, −10 box [ATAAAC]) and a putative ribosome-binding site (GGA; calculated free energy [ΔG] = −9.4 kcal) located 5 bp ahead of the start codon. In addition to the putative transcriptional signals, two other pro-

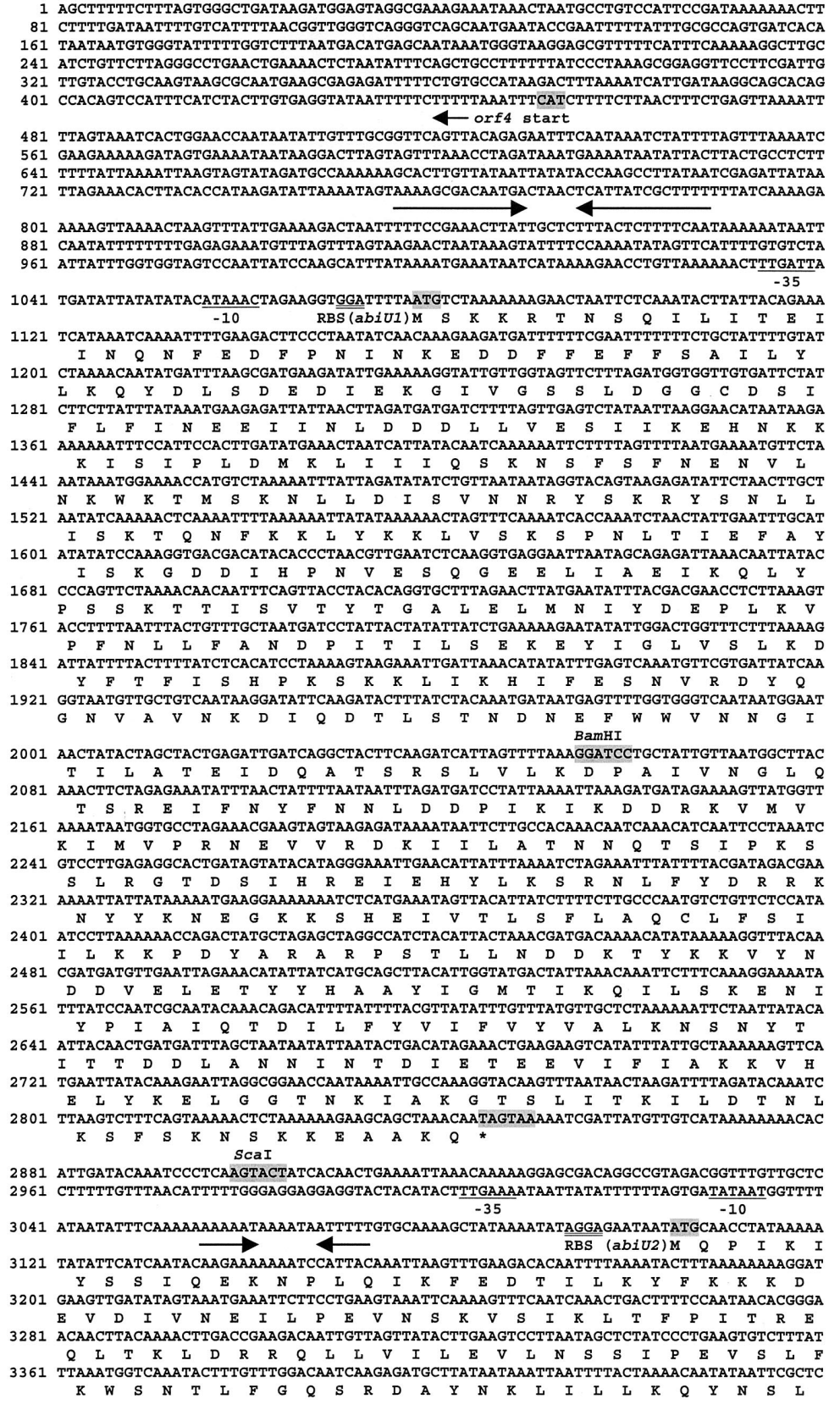

FIG. 2. Nucleotide sequence of the insert in pND002 and the deduced amino acid sequence. The deduced amino acid sequences are shown below the nucleotide sequence. Indicated are the putative ribosome-binding sites (RBS) (double underline), −35 and −10 promoter sequences (single underline), stop codons (asterisks), and possible transcriptional terminators (converging arrows), start and stop codons, and restriction enzyme sites (shading).

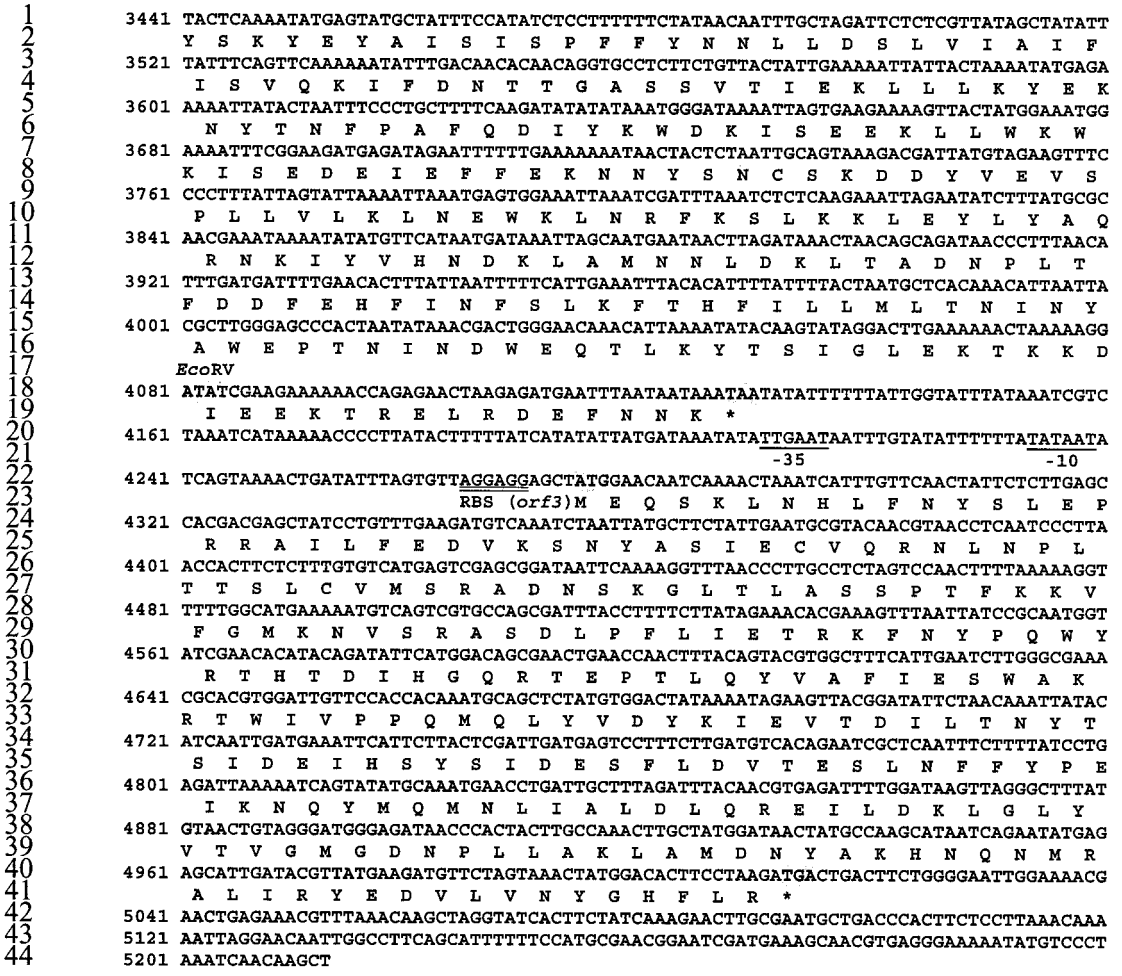

FIG. 2—*Continued.*

moter-like structures (−35 and −10 box) were identified upstream of *abiU1*. *abiU2* is 1,023 bp in length, starting at bp 3105 with AUG as the start codon and ending at bp 4127, followed by one stop codon (UAA). It has a G+C content of 25% and encodes a predicted protein of 40.6 kDa. Upstream of *abiU2* are a consensus −10 box (TATAAT) and a partial consensus −35 box (TTGAAA) separated by 20 bp. A putative ribosome-binding site (AGGAG) with a ΔG of −12.8 kcal is located 6 bp upstream of the *abiU2* start codon. *orf3* starts at bp 4275 and ends at bp 5012. It is 737 bp long and has a G+C content of 37%. A consensus −10 region (TATAAT) was identified 35 bp upstream of this ORF. At 17 bp upstream of this −10 region there is a typical −35 region (TTGAAT). A typical ribosome-binding site was also identified 4 bp ahead of the ATG start codon (AGGAGG). No ρ-dependent or ρ-independent terminator was identified between *abiU1* and *abiU2* or downstream of *abiU2*. No significant homology with *abiU1* was found at the protein level when searched in NR proteins by BLASTP and FASTA. No transmembrane regions were detected by using the DAS program (5), thus suggesting that AbiU1 is a cytoplasmic protein.

The nucleotide and protein sequences resulting from the 5.2-kb fragment were compared to the nonredundant nucleotide and protein databases by using the BLAST and FASTA programs. The amino acid sequence from positions 25 to 201 of the AbiU2 protein has 26% identity and 43% similarity with the predicted protein of AbiGii (26). The DNA sequence from bp 1 to 849, which includes the truncated *orf4* (*orf4* starts at bp 453 and is read into the opposite direction), was found to have 98% identity with the DNA sequence of the *L. lactis* ATP binding protein (GenBank accession numbers M90969, M77093, and M98400). The derived amino acid sequence of *orf4* has 97% identity with the same protein. The protein encoded by *orf3* was found to have 72% identity with the derived product of *orfU*, the function of which is unknown. The *orfU* gene is adjacent to *abiF* in plasmid pNP40 (13).

Identification of the ORF(s) that encodes phage resistance in pND002. To determine what is responsible for the phage-resistant phenotype, two deletion plasmids were constructed. *abiU1* and *orf4* were deleted, resulting in the pND003 (Fig. 3). To study the phenotype of *abiU1*, *abiU2* and *orf3* were removed by constructing pND006 (Fig. 3). Cross-streaking with ϕ712 and ϕc2 showed that LM0230(pND003) was sensitive to both phages, but LM0230(pND006) was resistant to both (Ta-

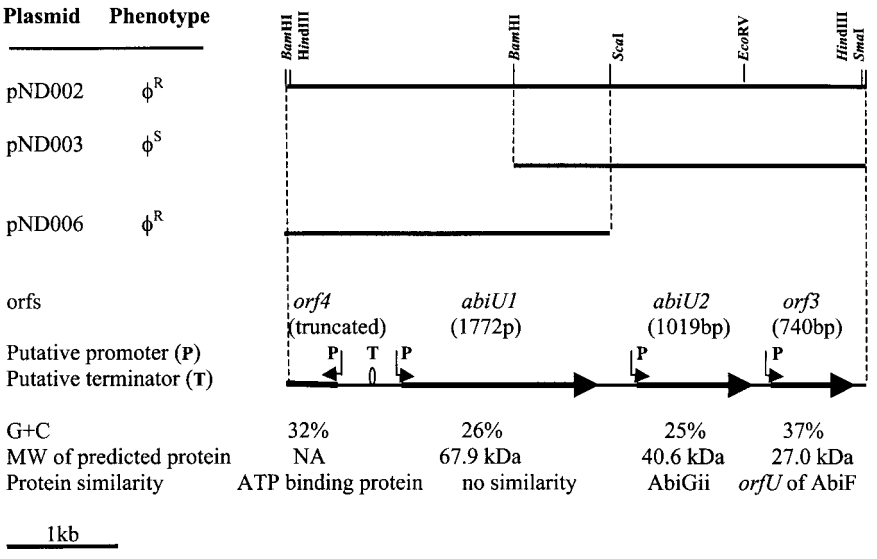

FIG. 3. Gene organization of the 5.2-kb insert in pND002 and the subcloned DNA fragments.

ble 2). These results suggest that *abiU1* is the primary phage resistance determinant in pND002.

Possible downregulation function of AbiU2 on AbiU1. The degree of phage resistance obtained with pND002 and pND006 was compared (Table 2). With ϕ712, the EOP obtained on LM0230(pND006) was much lower than that obtained with LM0230(pND002) (10^{-4} versus 10^{-2}). When the same experiment was repeated with ϕc2, however, there was very little difference observed between the EOPs obtained with LM0230(pND006) and LM0230(pND002) (Table 2).

To determine whether the difference observed between LM0230(pND006) and LM0230(pND002) was evident against the P335 phage species, the same experiment was performed with ϕul36. Plasmids pND002 and pND006, as well as the vector pDL278, were electroporated into *L. lactis* UL8, which is the host for ϕul36. A similar phenomenon to that reported with ϕ712 was observed: the EOP obtained with UL8 (pND006) was 10^{-1} lower than that obtained with UL8(pND002) (Table 2). It should also be noted that the sizes of the ϕul36 plaques on UL8(pND006) were smaller than those obtained on UL8(pND002). The cell survival rate after ϕul36 infection of the two hosts UL8(pND002) and UL8(pND006) was similar to the sensitive host UL8(pDL278), further indicating that *abiU* is an abortive infection system.

The data suggest that this abortive infection system acts differently on phage from different species. The presence of *abiU1* in pND006 confers a low EOP against all phage species. In comparison to pND006, the presence of the complete 5.2-kb fragment in pND002 actually reduces phage resistance against 936 and P335 species but does not affect the phage resistance observed with ϕc2 (Table 2). The presence of the sequence downstream of *abiU1* appears to downregulate phage resistance in pND002. Given the homology of the AbiU2 protein to AbiGii, it is tempting to speculate that this protein may be responsible for this effect.

Effect of pND002 on phage DNA transcription. To study the effect on phage ϕ712 transcription, total RNA was isolated from LM0230(pDL278), LM0230(pND002) and LM0230 (pND006) at 10-min intervals after ϕ712 infection and Northern blotted. The blot was probed with labeled genomic DNA from ϕ712 (Fig. 4). Transcripts of ϕ712 first appeared 10 min after infection of strains LM0230(pDL278) and LM0230 (pND002). Transcripts were first detected in LM0230 (pND006) 30 min after infection, and the degree of transcription increased by 40 min. Plasmids pND002 and pND006 appear to delay phage transcription relative to the control strain.

The effect of pND006 on ϕc2 RNA transcription was also investigated. Samples were withdrawn 0, 45, and 75 min after ϕc2 infection and analyzed by Northern hybridization by using labeled genomic DNA of ϕc2 (Fig. 5). Phage c2 transcripts were detected 45 and 75 min after infection in the sensitive host LM0230(pDL278) but not in the resistant host LM0230(pND006). Apparently, the presence of pND006 significantly reduced or delayed ϕc2 RNA synthesis.

DISCUSSION

In this study, aspects of the molecular characterization of a lactococcal phage abortive infection system (AbiU) have been described. AbiU was isolated from the plasmid pND001 found in the industrial strain *L. lactis* LL51-1. pND001 conferred resistance to small isometric-headed ϕ712 (936 species) and prolate-headed ϕc2 (c2 species). EOP tests of LM0230 (pND001) and LM0230(pND002) on ϕc2 showed a similar level of resistance from both plasmids. However, EOP values of LM0230(pND001) to ϕ712 were 2 orders of magnitude less than that of LM0230(pND002) (Table 2). This suggests that another mechanism of phage resistance against ϕ712 exists or that the existing mechanism is enhanced on pND001 but not on pND002.

The phage resistance phenotype conferred by pND002 was characterized. Plasmid pND002 conferred reduced plaque size (Table 2), EOP (Table 2), burst size (Fig. 1), and ECOI against ϕ712. Plasmid pND002 conferred reduced plaque size and

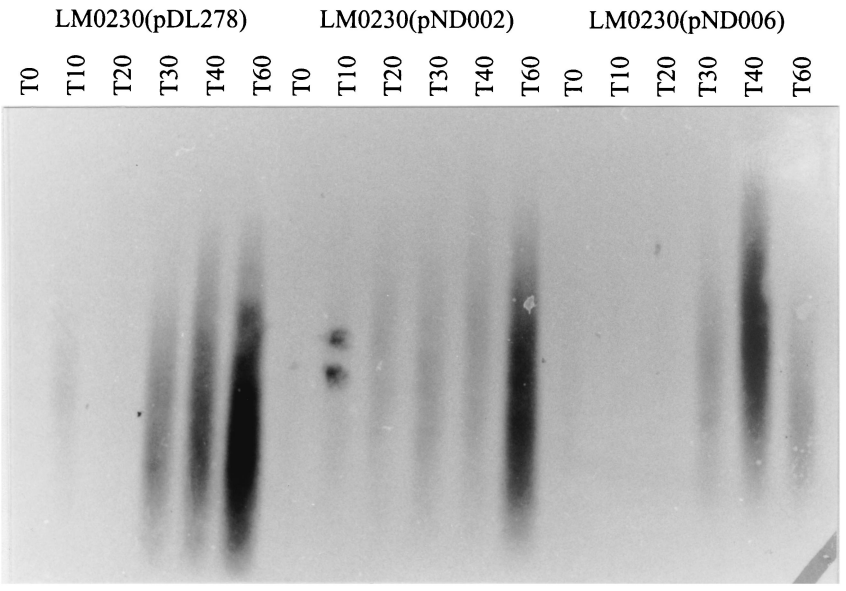

FIG. 4. Hybridization of total RNA isolated from ϕ712-infected *L. lactis* hosts by using ϕ712 genomic DNA as the probe.

EOP against ϕc2 and ϕul36; this plasmid prevented lysis of the host but conferred a very low survival rate against all three phage tested. These observations are typical of those observed with abortive infection systems.

Analysis of the 5.2-kb fragment in pND002 revealed the presence of three complete ORFs and one incomplete ORF. Cloning experiment were used to determine that *abiU1* is directly involved in phage resistance. Comparison of the EOPs obtained with pND002 and pND006 against phages ϕ712 and ϕul36 suggest that sequences downstream of *abiU1* interfere with or downregulate phage resistance. Like other *abi* genes (12), both *abiU1* and *abiU2* have low G+C contents of 26 and 25%, respectively. In contrast, the G+C content of the truncated *orf4* is 32%, and that of *orf3* is 37%, values which are close to the average G+C content of lactococcal chromosome. The homology of the AbiU2 and AbiGii proteins adds support to the hypothesis that *abiU2* is involved in phage resistance. Stronger resistance to ϕ712 and ϕul36 was conferred by pND006 relative to pND002, suggesting that *abiU2* or its protein might be involved in *abiU1* or AbiU1 downregulation (Table 2). This downregulation function of *abiU2* on *abiU1* seems to affect resistance against only isometric-headed phage from the 936 and P335 species and has no effect on prolate-headed phage from the c2 species.

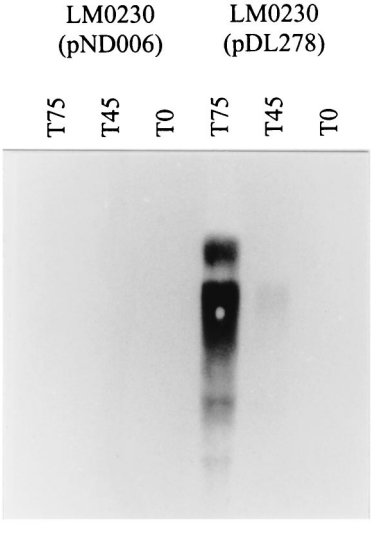

FIG. 5. Hybridization of total RNA isolated from phage c2-infected hosts by using ϕc2 genomic DNA as the probe.

The possible downregulation function of AbiU2 on AbiU1 resembles the negative control of PifC on *pif* gene expression in F exclusion of phage T7 in *E. coli* cells (23). The Pif system, located on the F plasmid in *E. coli*, is one of the abi systems identified in *E. coli* and confers host resistance to phage T7 infection. The *pif* region contains at least three genes—*pifC*, *pifA*, and *pifB* (29)—in which *pifA* encodes phage resistance. It is known that *pifA* and *pifC* lie within a polycistronic operon (4) and that the promoter is upstream of *pifC*. Construction of fusion proteins of PifA-LacZ, PifB-LacZ, and PifC-LacZ showed that *pifC* expression is autoregulated. PifC, in *trans*, significantly decreases the level of β-galactosidase activity produced by PifA-LacZ, PifB-LacZ, and PifC-LacZ. In addition, inactivating *pifC* in *cis* dramatically increased the resistance to T7 conferred by PifA, just as deleting *abiU2* increases the phage resistance conferred by AbiU1. Interestingly, PifC is a 40-kDa protein (23), which is similar in size to the predicted 40.6 kDa of AbiU2, whereas PifA is a 70-kDa protein (29), a size similar to the predicted 67.9 kDa of AbiU1. The DNA of the *pif* region has not been sequenced to allow a more detailed comparison.

One important step in understanding the mechanism of an abortive infection system is to locate the stage at which it inhibits the phage life cycle. Detecting phage mRNA transcrip-

tion by Northern hybridization demonstrated that AbiU appeared to delay transcription of both φc2 and φ712. The effect was more extreme for φc2 (Fig. 4 and 5). However, the Northern hybridization signals that were observed were not discrete. Likewise, O'Connor et al. also observed similar signals when studying AbiG and φsk1 (26). These authors tentatively explained it as a result of nonspecific processing of transcripts, transcript degradation, the presence of fragments of increasing size with a common 5′ end, or some combination of these factors.

Among the three lactococcal mechanisms reported to inhibit or delay phage transcription (16, 26, 27), AbiU resembles most closely pBu1-8 (16). However, since pBu1-8 is a native plasmid and the phage resistance determinant was not subcloned, it is not known whether other phage resistance systems exist on pBu1-8 that may provide additional phage resistance. On the basis of sequence homology, AbiU appears related to AbiG, which is part of another mechanism that interferes with phage transcription.

The phage resistance spectrum of an abi system is varied. Most abi systems confer resistance to one or two phage species, whereas only AbiA and AbiK have been reported to confer resistance to three species of phage: c2, 936, and p335 (11, 19). AbiU is the third abi system that encodes resistance against representative phages from all three of these phage species.

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