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Lytic Infection of *Lactococcus lactis* by Bacteriophages Tuc2009 and c2 Triggers Alternative Transcriptional Host Responses

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Here we present an entire temporal transcriptional profile of *Lactococcus lactis* subsp. *cremoris* UC509.9 undergoing lytic infection with two distinct bacteriophages, Tuc2009 and c2. Furthermore, corresponding high-resolution whole-phage genome tiling arrays of both bacteriophages were performed throughout lytic infection. Whole-genome microarrays performed at various time points postinfection demonstrated a rather modest impact on host transcription. The majority of changes in the host transcriptome occur during late infection stages; few changes in host gene transcription occur during the immediate and early infection stages. Alterations in the *L. lactis* UC509.9 transcriptome during lytic infection appear to be phage specific, with relatively few differentially transcribed genes shared between cells infected with Tuc2009 and those infected with c2. Despite the apparent lack of a coordinated general phage response, three themes common to both infections were noted: alternative transcription of genes involved in catabolic flux and energy production, differential transcription of genes involved in cell wall modification, and differential transcription of genes involved in the conversion of ribonucleotides to deoxyribonucleotides. The transcriptional profiles of both bacteriophages during lytic infection generally correlated with the findings of previous studies and allowed the confirmation of previously predicted promoter sequences. In addition, the host transcriptional response to lysogenization with Tuc2009 was monitored along with tiling array analysis of Tuc2009 in the lysogenic state. Analysis identified 44 host genes with altered transcription during lysogeny, 36 of which displayed levels of transcription significantly reduced from those for uninfected cells.

Bacteriophages are biological entities that function as obligate parasites; they cannot multiply without the biosynthetic functions supplied by their bacterial hosts. In order to proliferate, they must infect their host strain(s) to produce progeny, which, in the majority of cases, results in cell death through lysis and the release of new virions (1). Alternatively, temperate bacteriophages are able to undergo lysogenic infection, whereby lytic functions are repressed and the viral genome integrates into the host chromosome (2). Some temperate phages impart a lysogenic conversion phenotype to their hosts, whereby the hosts benefit from lysogenic infection. This benefit can take the form of superinfection exclusion or virulence phenotypes (3).

Phage-host interactions have been thoroughly detailed in *Escherichia coli* and have been explored in a handful of other species (4). Generally, host gene transcription is inhibited, either by phage-encoded inhibitors of the host's RNA polymerase, as demonstrated for T7-like and ϕKMV-like phages (the latter infecting *Pseudomonas aeruginosa*) (5, 6), or by the modification of host RNA polymerase to redirect transcription exclusively toward phage genes, as in the case of phage T4 (7). In addition, T4 phage-encoded ADP-ribosyltransferases interact with host-encoded proteins, some of which are involved in translation processes (8). Recently, Lavigne and colleagues (9) have identified possible phage-directed posttranslational modifications of host proteins as another level of host modulation during lytic infection of *P. aeruginosa*.

Whole-genome transcriptomics studies have shown that host transcription shutdown or reprogramming is by no means a universal feature of bacteriophage infection (10–14). The majority of these studies, undertaken in *E. coli*, *P. aeruginosa*, *Lactococcus lactis*, and the archaeon *Sulfolobus solfataricus* , have revealed a remarkably small effect of phage infection on host transcriptomes, with more-pronounced changes occurring only during late infection and usually involving increased transcription of stress response-associated genes.

Analysis of the lactococcal host transcriptome during the early stages of phage infection has recently been carried out by Fallico and colleagues (11). The host response of *Lactococcus lactis* subsp. *lactis* IL1403 infected with phage c2 was monitored 10 min postinfection. Sixty-four genes displaying levels of transcription different from those for uninfected cells were identified. From these data, the authors hypothesized a complex four-strand host response to c2 infection, which targets membrane stress proteins, decoration of cell wall polysaccharides, energy conservation, and maintenance of the proton motive force.

L. lactis is used globally as a starter culture for cheese production. Bacteriophages infecting *L. lactis* can lead to slow or failed fermentations, negatively affecting the production process, as well as the quality of the product, and are therefore a major economic concern (15). Due to the scale of this problem, bacteriophages infecting lactic acid bacteria (LAB), such as *L. lactis*, have been thoroughly characterized, especially with regard to bacteriophage genomics and host defense mechanisms (16, 17).

Lactococcal phages are grouped into 10 recognized species (18)

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on the basis of morphology, genetics, and infection characteristics. Of these, 3 species are routinely isolated from dairy fermentations (19). These include the 936 species (small isometric headed), the c2 species (prolate headed), and the highly polythetic P335 species (small isometric headed). All three species are members of the double-stranded DNA (dsDNA) *Caudovirales* order and belong to the family *Siphoviridae*, possessing long, noncontractile tails. Apart from members of the P335 species, which can be lysogenic, these phages are strictly lytic.

L. lactis UC509.9, whose complete genome has recently been made available (20), is an Irish dairy starter strain isolated from a mixed starter culture in the 1980s (21). *L. lactis* UC509.9 is a lysogenic and lytic host to the well-characterized P335-species phage Tuc2009 (21–24) and is also able to undergo infection by the strictly lytic c2 species phage, which has been thoroughly characterized (25–27). Monitoring the host transcriptional response to infection by different bacteriophages will help us understand how bacteria cope with bacteriophage infection. In the current study, we demonstrate that infection of *L. lactis* UC509.9 by either c2 or Tuc2009 has a rather modest impact on host transcription, yet our results reveal a phage-specific host response to infection.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and growth conditions. *L. lactis* UC509.9 and the Tuc2009-lysogenized derivative of *L. lactis* UC509.9, *L. lactis* UC509, were grown under static conditions in GM17 broth (M17 broth [Oxoid Ltd., Hampshire, United Kingdom] supplemented with 0.5% D-glucose). Bacteriophages c2 (26) and Tuc2009 (21) were propagated on *L. lactis* UC509.9 as described previously (28) and were maintained at 4°C. Bacteriophages were concentrated using 10% polyethylene glycol, as described previously (29).

DNA microarray and tiling array analysis. DNA microarrays containing oligonucleotide primers representing each of the 2,066 annotated genes on the genome of *L. lactis* UC509.9 (GenBank accession number CP003157), as well as two complete genome tiling arrays of bacteriophages Tuc2009 (NC_002703) and c2 (NC_001706) at 4-bp resolution, were designed using eArray (Agilent)and chipD (30) and were obtained from Agilent Technologies (Palo Alto, CA, USA).

For sample collection, 600 ml of prewarmed GM17 broth (30°C) was inoculated with 2% of an overnight culture of *L. lactis* UC509.9. This culture was grown at 30°C under static conditions to an optical density at 600 nm (OD_{600}) of 0.13, at which point $CaCl_2$ was added to a final concentration of 10 mM. The culture was further incubated for 10 min to allow equilibration. At this point, the culture was split into two equal volumes. To one portion, 10 ml of a concentrated 1.5×10^{10}-PFU/ml phage suspension (either c2 or Tuc2009) in TBT buffer (100 mM Tris [pH 7.5], 100 mM NaCl, 10 mM $MgCl_2$) was added, producing a final multiplicity of infection (MOI) of 5. To the other portion, used as a control, a corresponding amount of TBT buffer without phage was added. Samples (30 ml) were collected at 2, 5, 10, 15, 25, 35, and, for Tuc2009 only, 45 min postinfection (p.i.) by centrifugation (performed in a Thermo Scientific SL16R centrifuge using a 75003658 rotor at 5,500 rpm for 1 min). Pellets were flash frozen in a −80°C ethanol (EtOH) bath. Samples were then maintained at −80°C until further processing and analysis.

Cells were disrupted, and total RNA was extracted, as described previously (31) using a High Pure RNA isolation kit (Roche, Germany). RNA quality and yield were assessed by observation of band integrity on a 1% agarose gel and determination of the OD_{260}. For all RNA preparations, the OD_{260}/OD_{280} ratio was >1.9 and the OD_{260}/OD_{230} ratio was >2.1. For cDNA synthesis, 10 to 20 μg of total RNA was used in an annealing reaction with 1.6 ng μl^{-1} of random nonamers (MWG Biotech, Germany). For the tiling arrays, to prevent spurious second-strand synthesis, 0.1 μg μl^{-1} of actinomycin D (Sigma) was added in addition to 1 μl RNaseOUT (Invitrogen, USA). The annealing mixture was heated to 70°C for 5 min and was then cooled at room temperature for 10 min. Reverse transcription was performed using 10 μl of the annealing mixture and SuperScript III reverse transcriptase (Invitrogen, USA) for 16 h at 42°C. cDNA was purified using the Kreatech DSK-001 kit (Kreatech, Amsterdam, Netherlands). Twenty-five micrograms of cDNA was labeled with Cy3 or Cy5 by using the Kreatech DSK-001 labeling kit (Kreatech, Amsterdam, Netherlands). For the tiling array, samples containing bacteriophage cDNA were labeled with Cy3, while control samples were labeled with Cy5.

Labeled cDNA was hybridized using the Agilent Gene Transcription hybridization kit (part number 5188-5242) as described in the manual for Agilent two-color microarray-based gene expression analysis, version 4.0 (G4140-90050). Following hybridization, microarrays were washed in accordance with Agilent's standard procedures and were scanned using an Agilent DNA microarray scanner (model G2565A). The scanning results were converted to data files and were normalized with Agilent's Feature Extraction software (version 9.5) using default settings with the following modification: Loess normalization was performed using the probes representing *L. lactis* UC509.9 as a reference set, as in a previously described method (32). Differential transcription tests were performed with the Cyber-T implementation of a variant of the *t* test, as described previously (33). A gene was considered differentially transcribed if the *P* value was ≤0.001 and the ratio of the level of transcription to the level of transcription for the control was ≥2.5 (or ≥5 for lysogen experiments). For the tiling array analysis, interslide normalization was performed by determining a scaling factor per slide from the total signal of all UC509.9 probes and then dividing the signals of the tiling probes by the scaling factor. To determine transcription start sites (TSS), the melting temperature (T_m) of the portion of the tiling probe overlapping an area where the suspected transcript commenced was calculated. The TSS was determined to be at the position where the overlapping probe T_m was similar to the probe hybridization temperature.

qRT-PCR microarray validation. cDNA was generated as described above. Ambion Turbo DNase (Invitrogen, USA) was used to ensure that RNA samples were DNA free prior to reverse transcription. Primers for quantitative reverse transcription-PCR (qRT-PCR) were designed using Primer3Plus (34). qRT-PCR was performed using SYBR green I Master Mix (Roche, USA), and the reactions were performed according to the manufacturer's instructions, in triplicate, using a LightCycler 480 II detection system (Roche, USA). Cycling conditions for all amplifications consisted of an initial activation step of 95°C for 10 min, followed by 45 cycles of 95°C for 15 s, 55°C for 5 s, and 72°C for 15 s. Transcription levels were determined using the $2^{-\Delta\Delta CT}$ method (35); the housekeeping gene *alaS* was used to normalize results (11).

Determination of chromosome integrity by quantitative PCR (qPCR). Chromosome integrity after Tuc2009 infection was determined as described previously (9). *L. lactis* UC509.9 was infected with Tuc2009 and cells harvested as described above. Complete genomic DNA was extracted as described previously (29). Standard curves (data not shown) of DNA copy numbers were prepared using the pGEM-T Easy vector system (Promega, USA) with *alaS* and *orf56* as amplification targets for the *L. lactis* UC509.9 and Tuc2009 genomes, respectively. Reactions and cycling conditions for targets were performed as described above.

Microarray data accession number. All microarray and tiling array data determined in this study were deposited in NCBI's Gene Expression Omnibus (GEO) database (36) under the GEO series accession number GSE45655.

RESULTS

Transcriptional response of *L. lactis* UC509 to lysogeny compared with transcription by prophage-cured UC509.9. To determine the effects of Tuc2009 lysogeny on its host, a Tuc2009-(re) lysogenized derivative of *L. lactis* UC509.9 was obtained and was designated *L. lactis* UC509. Transcriptomes of *L. lactis* UC509 and

UC509.9, grown to early-exponential phase (OD_{600}, ~0.15), were obtained and compared. Analysis of microarray data demonstrated 44 chromosomal genes differentially expressed in *L. lactis* UC509 and UC509.9 (Table 1). Of these, the majority, i.e., 36 genes, exhibited lower transcription levels in UC509, while the remaining 9 genes exhibited higher transcription levels in UC509 than in its Tuc2009-free ancestor UC509.9. The downregulated genes encode proteins involved in nucleotide biosynthesis (*ntd*, *dukA*, *nudH*), amino acid metabolism (*hisB*, *trpAD*), and respiration (*menE*, *hemH*). In addition, four genes (represented by *uc509_0204*, *uc509_0210*, *uc509_0211*, and *uc509_0212*) presumed to be involved in the biosynthesis of the so-called lactococcal "pellicle," a cell wall polysaccharide the structure of which has been determined in *L. lactis* MG1363 (37), exhibited lower levels of transcription in the lysogenic strain than in prophage-cured UC509.9.

Of the eight chromosomal genes showing increased transcription during lysogeny, four encode transport proteins (*cadA*, *glpF2*, *uc509_1435*, *uc509_1436*). Of these, *uc509_1435* and *uc509_1436* encode members of the VIT1 protein family, which is involved in iron and manganese transport (38).

Transcriptional analysis of Tuc2009 during lysogeny. Transcriptional profiling of Tuc2009 during lysogeny was carried out by tiling array analysis in parallel with monitoring of the *L. lactis* UC509 response to lysogeny. In general, tiling array analysis displayed the expected profile for phages in the lysogenic state (Fig. 1). Transcription of cI_{2009}, which encodes the Tuc2009 repressor, responsible for maintaining the lysogenic state (2), and sie_{2009}, which specifies a superinfection exclusion protein, preventing infection by particular 936-species bacteriophage (28, 39), was clearly visible (Fig. 1). Previously, two transcripts, Y1, which covered sie_{2009} only, and Y2, which encompassed cI_{2009}, sie_{2009}, and int_{2009}, were detected by Northern blot analysis and were mapped to promoters upstream of sie_{2009} and int_{2009} (23). In our data, the Y1 transcript is not discernible, likely due to read-through of the Y2 transcript. The previously determined transcriptional start site (TSS) for Y2 corresponds well with our tiling data. No obvious transcriptional activity was observed for the integrase-encoding gene int_{2009} during lysogeny. The intergenic region between sie_{2009} and int_{2009} contains both a possible transcriptional terminator (positions 1249 to 1291), as predicted using Transterm (40), and possible −10 (TAAACT) and −35 (TTGACG) sequences that could constitute a promoter.

An unexpected observation was the transcription of *orf10*, which encodes a protein with no known function, during lysogeny. Low-level transcription was detected from a region beginning at a predicted TSS 27 bp upstream of *orf10*. Analysis using BPROM (SoftBerry) suggests a previously undetected promoter upstream of *orf10* (with proposed −35 and [extended] −10 sequences that correspond to ATGTAT and TGCTATAAT, respectively) and a predicted TSS at nucleotide position 4987 (GenBank accession number NC_002703). The genomic position of *orf10* is the exception to the general arrangement of Tuc2009 genes in two oppositely orientated clusters, for either a lytic or a lysogenic lifestyle; *orf10* is located within the early replication region in the opposite orientation to the surrounding genes. The GC content of *orf10* is 34.1%, similar to that of the rest of the replication module and typical of lactococcal phages and their hosts (23). The predicted product of *orf10* does not contain any domains discernible by BLASTP (41), Pfam (42), and HHpred (43) analysis, and therefore, we could not assign any function to this gene.

Bacteriophage tiling arrays during lytic infection. Temporal transcriptional profiling of Tuc2009 and c2 lytically infecting *L. lactis* UC509.9 was carried out using high-resolution tiling arrays. Previously, transcriptional analyses by Northern blotting and primer extension were performed in detail for both Tuc2009 (23) and c2 (27).

Of the six previously identified Tuc2009 transcripts, with determined start points, termed Y1, Y2, E1, E4, M1/L, and M2, only Y2, E1, and M1/L were clearly identifiable by tiling array analysis using mRNA isolated from Tuc2009-infected UC509.9 (Fig. 1). The Y2 transcript, previously predicted to encompass int_{2009}, sie_{2009}, and rep_{2009}, was detected at all postinfection time points (Fig. 1). However, as with Tuc2009 during lysogeny, this transcript appears to cease upstream of int_{2009}, 30 bp downstream of sie_{2009}. Only at 15 min postinfection can transcription of int_{2009} be observed. From our tiling data it is not possible to discern whether the transcript encompassing int_{2009} at 15 min postinfection is (a processed) part of the Y2 transcript or an individual transcript. The expression of these transcripts that encompass the lysogeny module was low compared to the expression of the lytic genes carried in transcripts E1 and M1/L.

The E1 transcript was detected at 2 min postinfection, with signal intensity increasing at 5, 10, and 15 min postinfection, before its strength decreased at subsequent time points, although the transcript was still detectable during late infection. The M1/L transcript was clearly visible from 25 min postinfection and persisted until the end of infection, in agreement with previous results (23). The start position(s) of the E4 and M2 transcripts could not be detected, although these may have been masked due to possible high read-through of the preceding transcripts.

The transcription patterns of phage c2 during *L. lactis* UC509.9 infection were also monitored using tiling arrays (Fig. 2). As in a previous study using *L. lactis* MG1363 as a host (27), c2 transcripts were detected from all early promoters at all postinfection time points. Transcription directed by the late promoter was first detected 10 min postinfection and appeared to correspond to a transcript that encompassed *l1* to *l10*, the latter encoding the predicted tail adsorption protein (26). The transcription pattern for *l6*, encoding a putative hypothetical protein, does not correlate with the transcription pattern for a functional gene. The transcript signal within *l6* is interrupted several times, decreasing at around position 11402 (GenBank accession number NC_001706.1) before increasing and decreasing over the remainder of *l6*. No transcription signal was detected for *l15*, which encodes a host specificity determinant (44). No obvious explanation can be given for this finding, although the c2 phage in our collection may have an alternative host specificity determinant, different from that for the c2 phage sequenced and deposited in GenBank. In all cases, for both bacteriophages, the transcriptional start sites that had been identified previously by primer extension analysis were shown to be consistent with the transcription initiation points determined by our tiling data (23, 27) (data not shown).

General features of the *L. lactis* UC509.9 response to Tuc2009 lytic infection. To examine the temporal transcriptional response of *L. lactis* UC509.9 to lytic infection, Tuc2009 was added to early-log-phase cells at an MOI of 5. Samples for RNA extraction were then harvested at regular intervals until 45 min postinfection. To determine the integrity of the genomic DNA of *L. lactis* UC509.9

TABLE 1 *L. lactis* UC509 genes showing levels of transcription significantly different[a] from those for Tuc2009 prophage-cured *L. lactis* UC509.9

COG category[b] and gene	Function[c]	Fold change	*P*
Amino acid transport and metabolism			
trpA	Tryptophan synthase α-chain	−5.7	7.71E−08
trpD	Anthranilate phosphoribosyltransferase	−8.9	8.79E−07
Carbohydrate transport and metabolism, *glpF2*	Glycerol uptake facilitator protein	5.8	3.56E−08
Cell wall/membrane/envelope biogenesis			
uc509_0204	Glycosyltransferase	−5.7	6.24E−11
uc509_0210	Glycosyltransferase	−5.1	2.49E−05
uc509_0211	β-1,3-Glucosyltransferase	−6.2	3.68E−05
Coenzyme transport and metabolism			
uc509_0212	Lipopolysaccharide cholinephosphotransferase	−7.5	8.78E−06
nadD	Nicotinate-nucleotide adenylyltransferase	−5.5	4.70E−05
hemH	Ferrochelatase	−5.5	8.35E−14
Energy production and conversion			
nifJ	Pyruvate-flavodoxin oxidoreductase	26.1	3.10E−06
uc509_0612	Inorganic pyrophosphatase	−5.4	3.04E−07
uc509_1669	Oxygen-insensitive NAD(P)H nitroreductase	−12.9	4.20E−05
Function unknown			
uc509_1435	Putative VIT family iron transporter	8	2.25E−09
uc509_1436	Putative VIT family iron transporter	5.8	5.78E−12
General function prediction only			
uc509_1021	Phosphohydrolase	−5.6	1.76E−07
uc509_1700	Hydrolase	−9.7	5.86E−09
Inorganic ion transport and metabolism			
cadA	Cadmium efflux ATPase	20.9	3.84E−08
amtB	Ammonium transporter	6.7	6.58E−05
uc509_0801	Arsenate reductase	−5.1	1.93E−06
cadD	Permease, cadmium resistance protein	−5.6	3.16E−06
Lipid transport and metabolism			
uc509_2209	Activator of (*R*)-2-hydroxyglutaryl-CoA dehydratase	12.1	9.16E−05
menE	*O*-Succinylbenzoic acid-CoA ligase	−5.1	3.20E−05
Nucleotide transport and metabolism			
ntd	Nucleoside deoxyribosyltransferase-I	−10.1	3.11E−08
dukA	Deoxyadenosine kinase	−6.5	5.78E−06
Posttranslational modification, protein turnover, chaperones			
uc509_1955	U32 peptidase	6.7	5.94E−09
tpx	Thiol peroxidase	−6.1	7.44E−06
Replication, recombination, and repair			
uc509_0068	Modification methylase ScrFII	−6	4.00E−06
nudH	Adenosine (5′)-pentaphospho-(5″)-adenosine pyrophosphohydrolase	−10.5	7.60E−07
xerD	Tyrosine recombinase XerD	−9.3	1.42E−06
Secondary-metabolite biosynthesis, transport, and catabolism, *uc509_1342*	rRNA (guanine-N1-)-methyltransferase	−13.1	2.81E−05
Transcription, *rmaD*	Transcriptional regulator, MarR family	−5.7	1.55E−09
Translation, ribosomal structure, and biogenesis			
uc509_0317	Transcriptional regulator, MarR family	−9.4	1.01E−11
uc509_0396	Transcriptional regulator, lysR family	−6.3	1.75E−06
uc509_0448	Transcriptional regulator	−10.4	1.17E−09
uc509_0583	Acetyltransferase	−6.6	1.73E−10
uc509_0706	Transcriptional regulator, MarR family	−5.5	5.01E−07
uc509_1191	Transcriptional regulator, MerR family	−5.3	1.12E−05
fbpA	Fibronectin/fibrinogen-binding protein	−9.9	8.46E−06
xylR	Xylose activator	−10.2	1.52E−05
uc509_1462	Transcriptional regulator, TetR family	−11.9	2.41E−07
uc509_2032	Transcriptional regulator, HTH type	−5.8	3.00E−06
uc509_2125	Transcriptional regulator, LacI family	−5.3	1.81E−06
rpmG	LSU ribosomal protein	−5.6	9.16E−07
uc509_1219	Aminoglycoside phosphotransferase	−6.5	3.33E−06

[a] At least 5-fold different; *P*, <0.001.
[b] COG, cluster of orthologous groups.
[c] VIT, vacuolar iron transporter; CoA, coenzyme A.

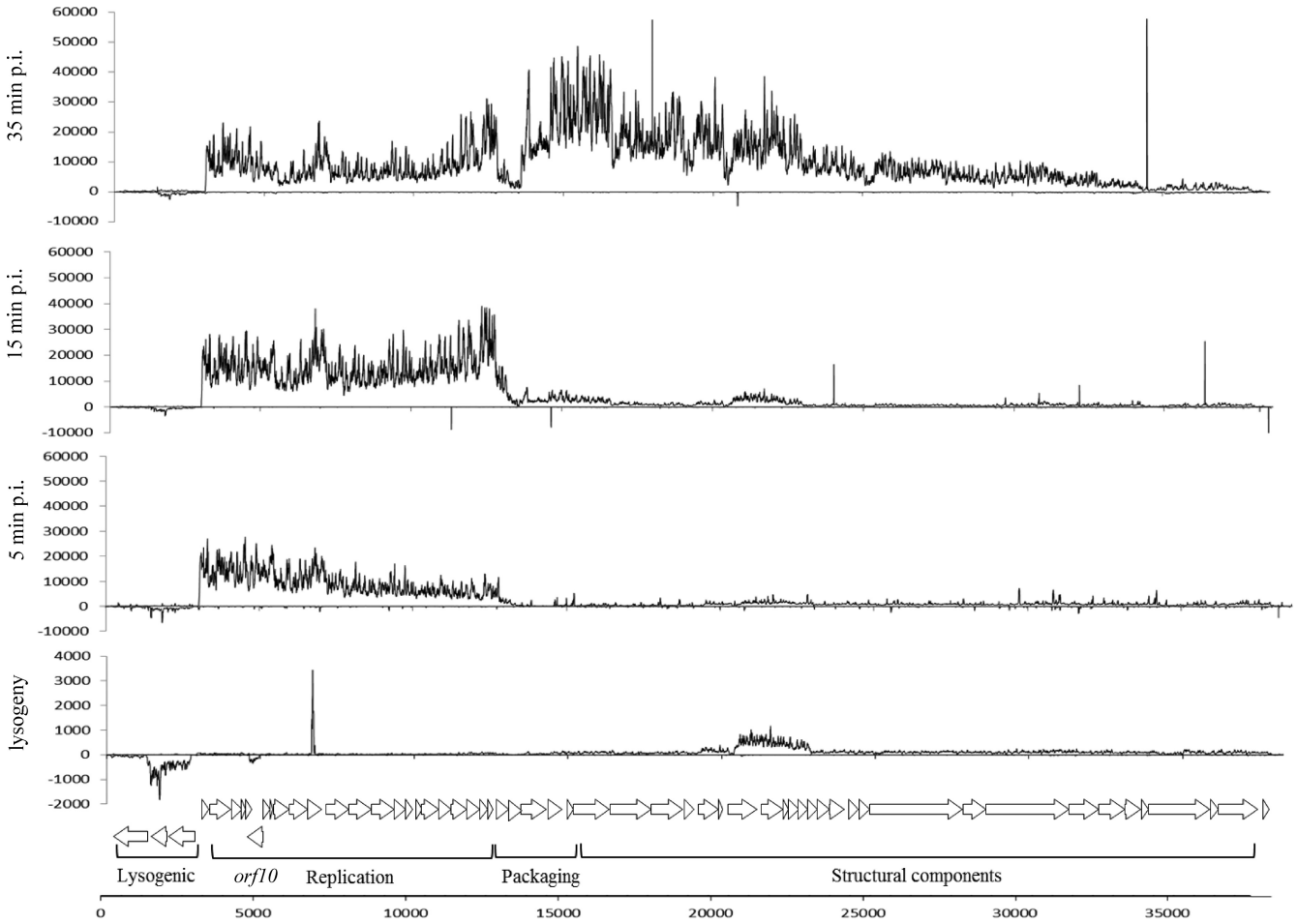

FIG 1 Tiling array signals of Tuc2009 infecting *L. lactis* UC509.9 at different stages postinfection (p.i.) and during lysogenic infection. To aid in the visualization of signals, scaling differs between plots. A schematic representation of the Tuc2009 genome, depicting putative protein-encoding regions, is shown at the bottom.

throughout infection, qPCR was performed at regular intervals postinfection. The host genome does not appear to be targeted for degradation, since copies of the host's *alaS* gene remain at a constant level throughout Tuc2009 infection (see Fig. S1 in the supplemental material). This is in line with expectations, since Tuc2009 has no gene whose product could provide such nuclease activity (23). Over the 45-min time course of Tuc2009 infection, 108 genes of the *L. lactis* UC509.9 genome exhibited transcription levels significantly altered from those of uninfected cells (at least 2.5-fold differential transcription; P, $\leq$0.001) (Fig. 3A). These represent approximately 5.5% of the total number of *L. lactis* UC509.9 genes identified (20). Of these 108 genes, 64 exhibited increased transcription, while 44 exhibited reduced transcription from that of uninfected cells. Of the 108 differentially transcribed genes, 55% showed differential transcription at more than one time point during the infection time course. No gene was observed to display a transcriptional level different from that in uninfected cells at all postinfection time points analyzed. During early Tuc2009 infection (i.e., 2 to 10 min postinfection), 19 genes were differentially regulated, compared to 109 genes at later time points (15 to 45 min postinfection). Microarray results were validated for selected genes at 35 min postinfection (Table 2), verifying that the microarray data obtained were a true reflection of phage infection-mediated differential transcription patterns.

Early Tuc2009 infection (2 to 10 min). During the early stages of *L. lactis* UC509.9 infection by Tuc2009, host genes involved in purine uptake, synthesis and salvage (*purM*, *add*, *pbuX*, and *xpt*) showed reduced transcription (Fig. 3A). *purM* is part of the well-characterized PurR-controlled regulon (45). While other PurR regulon genes do not show significant differential transcription by microarray analysis, qRT-PCR of *purK*, a member of the PurR regulon that encodes a phosphoribosylaminoimidazole carboxylase ATPase subunit, does suggest that other members of the PurR regulon also exhibit reduced expression during early infection (Table 2).

Increased transcription of *noxE*, encoding a NADH oxidase that enables the regeneration of NAD^+ from NADH in the presence of oxygen (46), at 5 min p.i. suggests increased energy production via glycolysis due to increased availability of NAD^+. This would result in an increase in pyruvate levels, which is reflected in the increased transcription of *ldh* (L-lactate-dehydrogenase) during late infection. Transcription of the *nifJ* and *uc509_2209* genes, whose products are functional under strictly anaerobic conditions, is also reduced during early infection (Fig. 3A).

Late Tuc2009 infection. More-pronounced differences between the transcriptomes of infected and uninfected cells occur from 15 min p.i. onward, with a total of 96 genes differentially transcribed during late infection (Fig. 3A). Only a small number of

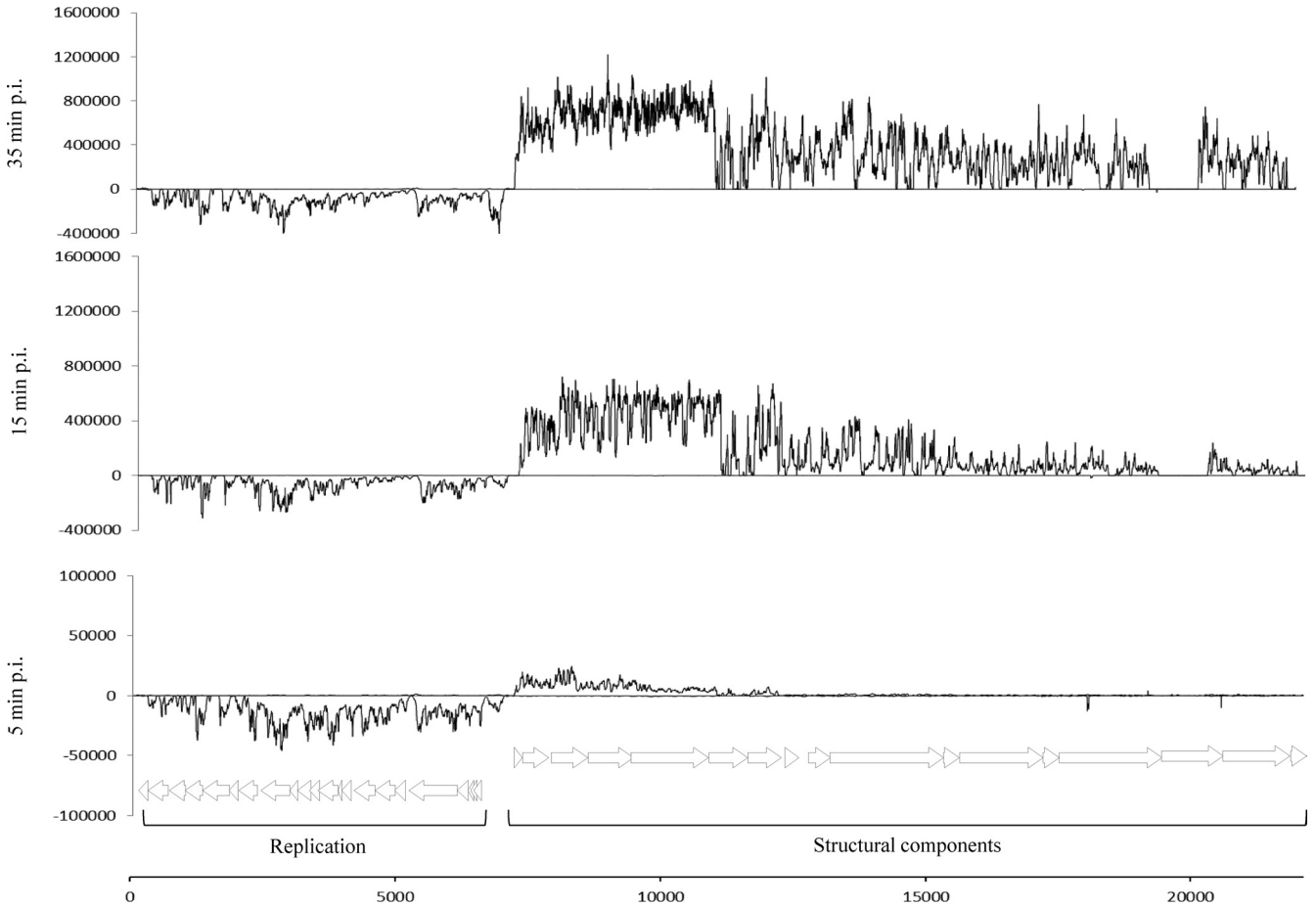

FIG 2 Tiling array signals of c2 infecting *L. lactis* UC509.9 at different stages postinfection. To aid in the visualization of signals, scaling differs between plots. A schematic representation of the c2 genome, depicting putative protein-encoding regions, is shown at the bottom.

genes that exhibited transcription levels different from those for uninfected cells during early infection remained differentially transcribed during late infection. The genes that continued to exhibit altered transcription (*nifJ*, *uc509_1358*, *uc509_1955*, *uc509_2146*) did so only until 25 min p.i.

The increased transcription of *ldh* (encoding L-lactate-dehydrogenase) during late infection suggests increased production of lactate from pyruvate. This process requires the reduction of NADH to NAD^+. As with the action of *noxE* during early infection, increased *ldh* activity would lead to increased availability of NAD^+ for energy production via glycolysis.

Genes involved in translation, including *argS*, encoding arginyl-tRNA synthetase, and *tgt*, encoding a tRNA guanine transglycosylase, show increased transcription from 25 to 45 min p.i. This may be a phage-mediated change, since high-level translation of the phage's structural proteins is occurring at this time.

Furthermore, *L. lactis* UC509.9 appears to modify its peptidoglycan structure during infection with Tuc2009. From 10 min p.i. through the remainder of the infection time course, *dacA*, encoding D-Ala-D-Ala carboxypeptidase, predicted to convert a pentapeptide side chain to a tetrapeptide side chain (47), exhibits increased transcription.

A significant increase in the transcription of *nrdGD*, specifying the anaerobic ribonucleotide reductase, also occurs during late infection. In addition, increased transcription of *nrdHIEF*, encoding the class Ib ribonucleotide reductase, which is functional under aerobic and anaerobic conditions (48), is also detectable. Ribonucleotide reductases catalyze the conversion of ribonucleotides to deoxyribonucleotides, thus making them available for DNA repair and replication. The increased expression of these genes is likely a response to diminished levels of nucleotides in infected cells relative to those in uninfected cells due to ongoing bacteriophage genome replication.

General features of the *L. lactis* UC509.9 response to c2 infection. During the 35-min time course of c2 infection, 157 genes of the *L. lactis* UC509.9 genome were shown to be transcribed at levels different from those for uninfected cells (at least 2.5-fold differential transcription; P, ≤ 0.001) (Fig. 3B). These represent approximately 7.5% of the *L. lactis* UC509.9 gene complement. Of these 157 genes, 108 exhibited increased transcription, while 49 exhibited reduced transcription from that in uninfected cells. Seventy genes exhibited differential transcription at more than one time point postinfection. No genes were differentially transcribed throughout infection, although *nrdGD*, encoding anaerobic ribonucleotide reductases, was downregulated from 5 min p.i. onward. The majority of differentially transcribed genes were detected toward the end (15 to 35 min) of infection. Of the 157 genes showing levels of transcription different from those for uninfected cells, 40 encoded (hypothetical) proteins of no known function.

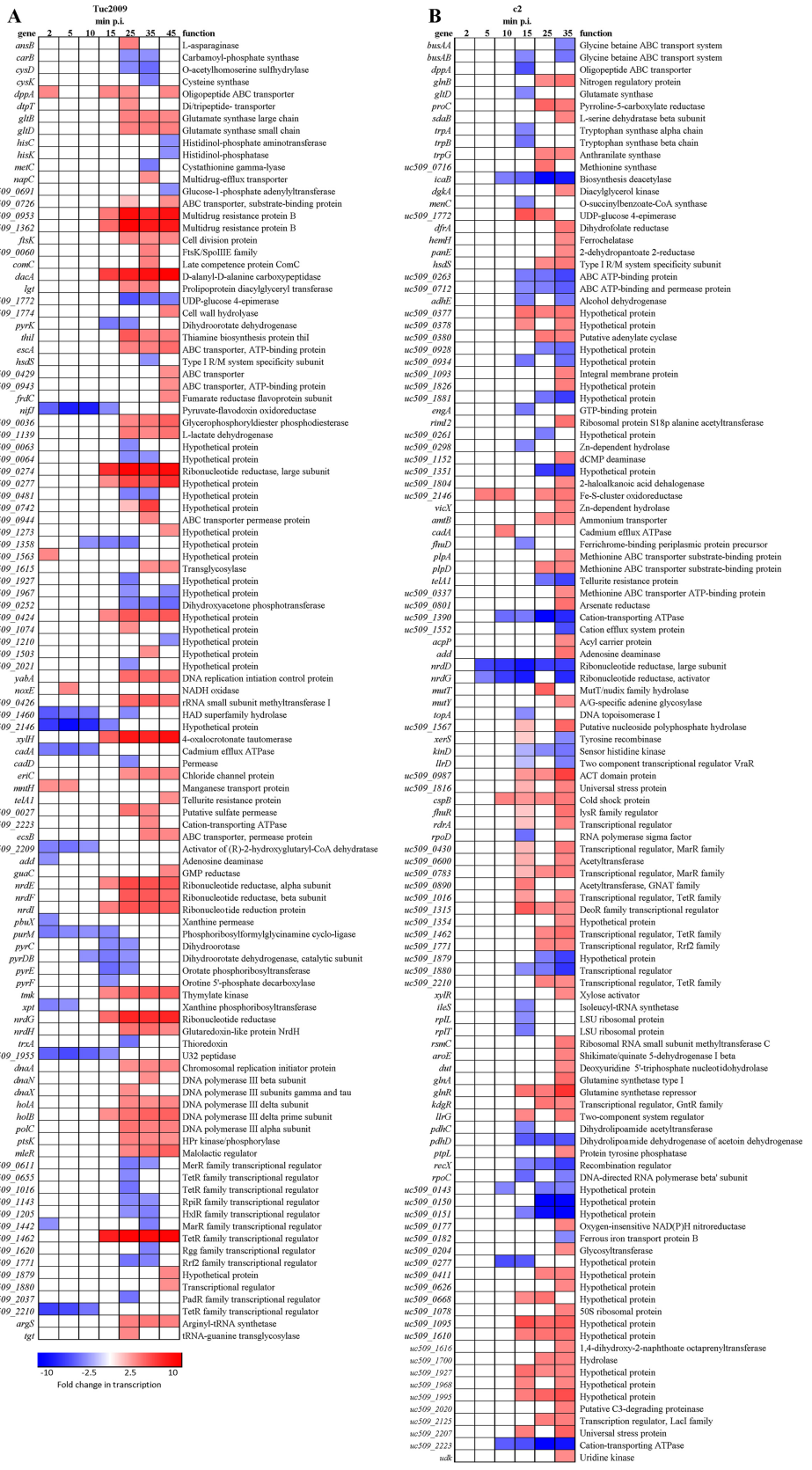

FIG 3 Heat maps of *L. lactis* UC509.9 genes significantly differentially transcribed (at least 2.5-fold differential transcription; P, <0.001) during lytic Tuc2009 (A) or c2 (B) infection at various times postinfection and in uninfected cells. The color scale represents the fold change in transcription (red, increased; blue, decreased).

TABLE 2 qRT-PCR validation of selected *L. lactis* UC509.9 genes displaying significantly altered transcription at 35 min after infection with Tuc2009

Gene	Function	Fold change by:	
		qRT-PCR	Microarray
argS	Arginyl-tRNA synthetase	6.6	3.12
dacA	D-Alanyl-D-alanine carboxypeptidase	5.6	7.46
holB	DNA polymerase III delta prime subunit	5.5	3.76
hsdS	Type I restriction-modification system, specificity subunit	2.2	2.40
metC	Cystathionine gamma-lyase	3.2	3.14
nrdD	Ribonucleotide reductase class III, large subunit	6.5	7.16
nrdE	Ribonucleotide reductase class Ib, α subunit	4.7	4.38
nrdF	Ribonucleotide reductase class Ib, β subunit	6.7	4.00
nrdG	Ribonucleotide reductase class III, activating protein	4.3	5.82
nrdI	Ribonucleotide reduction protein NrdI	5.0	4.08
purK	Phosphoribosylaminoimidazole carboxylase ATPase	5.3	NSD[a]
uc509_0426	rRNA small subunit methyltransferase I	2.5	3.66
uc509_0481	Hypothetical protein	17.5	2.58
uc509_1772	UDP-glucose 4-epimerase	2.5	3.25
xylH	4-Oxalocrotonate tautomerase	5.1	5.90
yabA	DNA replication initiation control protein	6.1	3.51

[a] NSD, not significantly different.

Early c2 infection. From the tiling array data (Fig. 2), transcripts directed by early c2 promoters were detected 2 min after infection of *L. lactis* UC509.9. During early infection (2 to 10 min p.i.), very few host genes showed differential transcription; only 10 of the 157 significantly differentially transcribed genes identified were detected during this period, and most of these encode hypothetical proteins with no known function. No *L. lactis* UC509.9 genes displayed differential transcription at 2 min p.i., while just 3 host genes (*nrdGD* and *uc509_2146*, predicted to encode subunits of a ribonucleotide reductase and an Fe-S cluster protein, respectively) were differentially transcribed at 5 min p.i. At 10 min p.i., 10 *L. lactis* UC509.9 genes, including the genes differentially transcribed at 5 min p.i., exhibited differential transcription. *icaB*, encoding a polysaccharide deacetylase, was shown to be downregulated from 10 min p.i. through the remainder of the time course.

Late c2 infection. Transcriptional activity originating from the late c2 promoter, PL1, can be detected in *L. lactis* UC509.9 after 10 min p.i. (Fig. 2); therefore, 15, 25, and 35 min p.i. are considered to be the late infection stages. During this time, 52, 34, and 122 host genes (at 15, 25, and 35 min p.i., respectively) displayed levels of transcription different from those for uninfected cells. The *nrdDG* and *icaB* genes, as mentioned above, continued to exhibit transcription levels lower than those for the uninfected control throughout late infection.

During late infection, differential transcription of genes involved in the metabolism of pyruvate is detected. Components of the pyruvate dehydrogenase complex (PDH), encoded by *pdhD* and *pdhC*, display levels of transcription lower than those for uninfected cells from 15 min p.i. onward. Furthermore, at 15 and 25 min p.i., *adhE* shows levels of transcription lower than those for uninfected cells. The reduction in the level of *adhE* transcription is likely due to the decreased abundance of acetyl coenzyme A (acetyl-CoA), produced by PDH. These alterations in the cellular catabolic flux suggest an increased tendency toward the homofermentative production of lactate, resulting in increased NAD^+ production.

Transcription appears to be limited during late infection, since *rpoC*, encoding the beta prime chain of RNA polymerase, exhibits reduced transcription levels at 15 and 35 min p.i., while *rpoD*, specifying the primary sigma factor for *L. lactis* (49), also displays transcriptional downregulation at 15 min p.i.

It is during late infection that genes involved in the stress response exhibit levels of transcription different from those in uninfected cells. The *uc509_1816* and *uc509_2207* genes, encoding proteins belonging to the universal stress response family (UspA) (50), show increased transcription at 15 min p.i. and at both 15 and 25 min p.i., respectively. Interestingly, genes involved in regulating the cell envelope stress response (CesSR) (51), encoded by *uc509_0928* and *uc509_0929* in *L. lactis* UC509.9, showed no differential transcription until 25 min p.i., when their transcription was diminished from that for uninfected cells.

Of the total number of differentially transcribed genes throughout the c2 infection time course, 77% (122 genes) are differentially transcribed at 35 min postinfection. At this time, various genes involved in amino acid biosynthesis and nitrogen metabolism (Fig. 3B) are upregulated. Since the cells are grown in a nutrient-rich medium (52), the elevated level of transcription over that for uninfected cells is unlikely to be due to nutrient limitation. A possible explanation is that the high level of bacteriophage protein production causes depletion of the intracellular supply of free amino acids.

Plasmid transcriptional response to Tuc2009 and c2 infection. *L. lactis* UC509.9 contains 8 plasmids, termed pCIS1 to pCIS8, encoding a total of 167 extrachromosomal genes, with functions required for the strain's adaptation to the dairy environment, such as lactose and casein metabolism (20). In keeping with the minimal effects on the host genome, only a small number of plasmid-borne genes displayed differential transcription during the infection time course with either phage: 19 and 33 genes showed alternative transcription with Tuc2009 and c2, respectively (Fig. 4). The majority of changes occurred during late infection, in genes that specify hypothetical proteins located on the largest plasmids, pCIS7 (52 kb) and pCIS8 (80 kb).

Of the phage defense mechanisms, only one gene, *pCIS8_051*, exhibited increased transcription 5 and 15 min following the initiation of c2 phage infection. This gene encodes a protein with high similarity to AbiB, an abortive infection protein previously shown to be active against c2 and 936-species phages (53). The fact that we can propagate c2 on *L. lactis* UC509.9 without difficulty suggests that our c2 phage is insensitive to AbiB. Differential transcription of the stress response-induced, low-fidelity polymerase encoded by *umuC* (54), 2 copies of which are present on pCIS7, occurs during Tuc2009 infection. The level of transcription of these two *umuC* genes is increased at 35 min p.i. During c2 infection, in contrast, they show alternative transcription relative to that of the uninfected control at 25 min p.i.

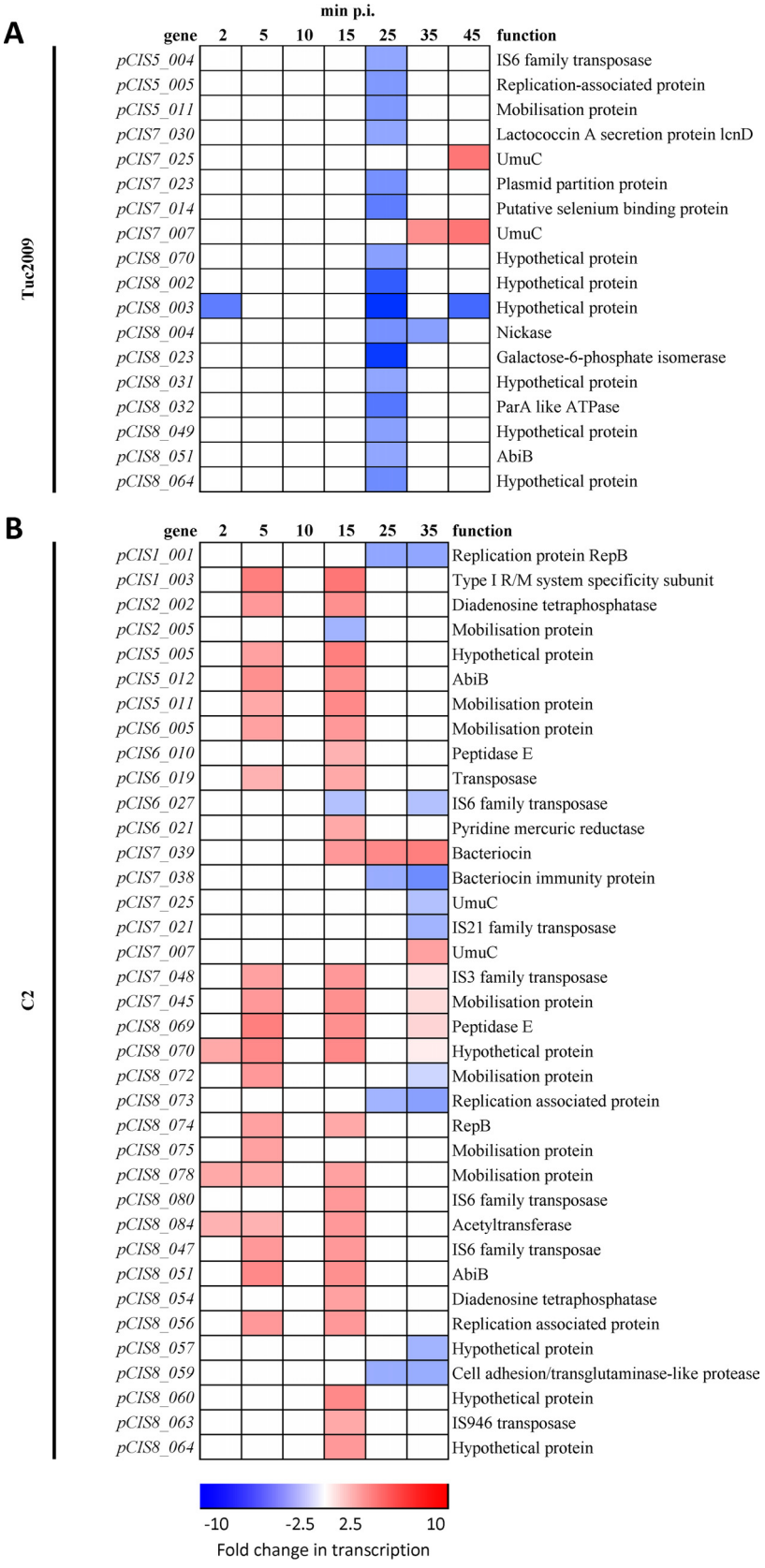

FIG 4 Heat maps of *L. lactis* UC509.9 plasmid genes significantly differentially regulated (at least 2.5-fold differential transcription; P, <0.001) during lytic Tuc2009 (A) or c2 (B) infection at various times postinfection and in uninfected cells. The color scale represents the fold change in transcription (red, increased; blue, decreased).

TABLE 3 *L. lactis* UC509.9 genes showing levels of transcription during either Tuc2009 or c2 infection significantly different[a] from those for uninfected cells

Gene	Product	Direction of transcriptional change[b] (min p.i.) for:	
		c2	Tuc2009
add	Adenosine deaminase	+ (35)	− (2)
cadA	Cadmium efflux ATPase	+ (10)	− (2, 5, 10)
dppA	Oligopeptide ABC transporter	− (15)	+ (2, 15)
gltD	Glutamate synthase [NADPH] small chain	− (15)	+ (25, 35, 45)
hsdS	Type I restriction-modification system, specificity subunit S	+ (35)	− (35)
nrdD	Ribonucleotide reductase of class III (anaerobic), large subunit	− (5, 10, 15, 25, 35)	+ (15, 25, 35, 45)
nrdG	Ribonucleotide reductase of class III (anaerobic), activating protein	− (5, 10, 15, 25, 35)	+ (15, 25, 35, 45)
telA1	Tellurite resistance protein	− (25, 35)	+ (45)
uc509_0277	Hypothetical protein	− (10, 15)	+ (15, 25, 35, 45)
uc509_1016	Transcriptional regulator, TetR family	+ (35)	− (25)
uc509_1462	Transcriptional regulator, TetR family	+ (35)	+ (15, 25, 35, 45)
uc509_1503	Hypothetical protein	+ (35)	+ (35)
uc509_1771	Rrf2 family transcriptional regulator	+ (25, 35)	− (25, 35)
uc509_1772	UDP-glucose 4-epimerase	+ (15, 25)	− (25, 35, 45)
uc509_1879	Hypothetical protein	− (25, 35)	+ (45)
uc509_1880	Transcriptional regulator	− (15, 35)	+ (45)
uc509_1927	Hypothetical protein	+ (15)	− (25)
uc509_2146	Hypothetical protein	+ (5, 10, 25, 35)	− (2, 5, 10, 15)
uc509_2210	Transcriptional regulator, TetR family	+ (35)	− (2, 5, 10)
uc509_2223	Cation-transporting ATPase	− (10, 15, 25, 35)	+ (35)

[a] Differences of >2.5 fold ($P < 0.001$).
[b] −, reduced transcription; +, increased transcription.

Comparative features of *L. lactis* UC509.9 infection by Tuc2009 and c2. Only 20 genes exhibited differential transcription during the infection of *L. lactis* UC509.9 by Tuc2009 as well as c2 (Table 3). Of these, only *uc509_1462* and *uc509_1503*, encoding predicted hypothetical proteins, displayed similar differential transcription during the two infections. The *uc509_1503* gene is predicted to encode a protein with five transmembrane helices that is upregulated at 35 min p.i. during c2 and Tuc2009 infection, while *uc509_2146*, encoding a hypothetical protein with flagellin *N*-methylase (FliB) family domains, displays increased transcription during early infection by both c2 and Tuc2009.

DISCUSSION

This study analyzed the temporal transcriptional response of *L. lactis* UC509.9 undergoing infection with either Tuc2009 or c2, representing phages of two different species (P335 and c2, respectively) of the family *Siphoviridae*. DNA microarrays of the host and high-resolution tiling arrays of each phage were used to provide corresponding data sets of the entire transcriptome at various points postinfection. An MOI of 5 was used in these experiments, which is not sufficient to infect every cell in the culture simultaneously yet is sufficient to ensure lysis of the experimental culture after 1 h, when viable phage particles are detected by plaque assay (data not shown). This level was applied because we did not want to place nonphysiological stress on the cells; high MOIs have been known to cause "lysis from without" (55) due to the muralytic enzymes associated with bacteriophage tails (56). It was presumed that there would be a subpopulation of uninfected cells in the phage-infected cultures at this MOI. We surmised that this would have minimal effect on our results, since the cells would behave as uninfected cells, apart from the possible dampening of the transcription profiles of genes in the infected samples.

Our data show that the response of *L. lactis* UC509.9 to infection is phage specific; relatively few differentially regulated genes are shared by transcriptomes obtained following infection by Tuc2009 and those obtained after infection with c2 (Table 3). Also, it was observed that infection with either phage has relatively minimal effects on the *L. lactis* UC509.9 transcriptome. Apparently, there is no need for major reprogramming of the cell at the transcriptional level in order to establish infection and produce phage progeny. The increased transcription of genes, many of which are involved in amino acid metabolism and nucleotide conversion, during late infection by both bacteriophages may be a cellular response to a perceived lack of metabolites due to depletion resulting from the rapid production of phage progeny.

The apparent lack of widespread cellular reprogramming or lack of a cellular transcriptional response has been noted previously for *E. coli*, where global changes in whole-genome transcription were moderate during infection with phage PRD1 (10). In the PRD1 model, most changes were shown to occur after bacteriophage synthesis, and the most highly induced host genes were stress related. It has been hypothesized previously that it may be in the interest of the phage not to elicit a defensive host response (10) and that this is therefore a specific strategy followed by the phage. It is worth noting that phages of the family *Tectiviridae*, of which PRD1 is a member, are not tailed phages and are genetically distinct from *Caudovirales* yet elicit apparently similar host responses. The minimal transcriptional response in *Lactococcus* species, and indeed in other species, also suggests that cellular adaptation for the production of phage progeny is likely to be more active at the translational or possibly the posttranslational level (9).

Despite the minimal impact on the host transcriptome and the apparent lack of a coordinated general phage response, Tuc2009 and c2 infections have three effects in common. These are (i) alternative transcription of genes involved in catabolic flux and

energy production, (ii) differential transcription of genes involved in cell wall modification, and (iii) differential transcription of genes involved in the conversion of ribonucleotides to deoxyribonucleotides. These effects are similar to responses observed by Fallico et al. (11), where genes involved in the decoration of cell wall polysaccharides and energy conservation displayed differential regulation during c2 infection of *L. lactis* IL1403. Studies of phage genome replication in the c2-related prolate-headed phage c6A suggest that phage genome replication continues until lysis (57). Therefore, the increase in the level of transcription of genes encoding ribonucleotide reductases is likely to enable continued rapid bacteriophage replication.

The alternative transcription of genes encoding proteins involved in cell wall modification during bacteriophage infection has been noted previously. Notably, *L. lactis* IL1403, when infected with c2, showed increased transcription of the *dltACBD* operon, responsible for D-alanyl modification of cell wall components (11). Moreover, modification of peptidoglycan has been linked to reduced susceptibility to 936-species phages in *L. lactis* MG1363 (58). There have also been suggestions that phage proteins target cell wall biosynthesis and modification in other species. Several phage-encoded proteins, such as Kil, encoded by the *E. coli* prophage Rac (59), have been shown to affect cell division. In the present study, during c2 infection, *icaB*, containing a polysaccharide deacetylase family domain (Polysacc_deac_1), exhibited reduced transcription from 10 min p.i., whereas during Tuc2009 infection, *dacA* transcription was increased. Knockout mutants of a polysaccharide deacetylase-encoding gene, *pgdA*, in *L. lactis* IL1403 exhibited fully acetylated peptidoglycan and increased autolysis (60), whereas knockout of *dacA* in *L. lactis* MG1363 caused increased lysozyme resistance (47). The increase in *dacA* transcription during bacteriophage infection may possibly be a Tuc2009-induced effect that aids in phage progeny escape.

Apart from perceived functional similarities, our results are remarkably different from those of the previous study by Fallico et al. (11), in which *L. lactis* IL1403 was infected with c2. Only 10 genes identified by Fallico and colleagues are differentiated during c2 infection in both studies. This is likely accounted for by the differences in experimental setup, such as the mid-log growth phase (OD_{600}, 0.4) and the very high MOI of 800 used in the previous study. Cellular physiology during mid-log phase would be markedly different from that of *L. lactis* UC509.9 during early-exponential phase (61). It is possible, therefore, that host infection profiles at different cell growth phases show alternative responses to phage infection.

In addition to the host response, we monitored the transcriptional profiles of both Tuc2009 and c2 throughout infection and that of Tuc2009 during lysogeny. Transcriptional profiles generally correlated with the findings of previous transcriptional studies of these two phages (23, 27). The most notable feature of the tiling array data is the detection of *orf10* transcription during lysogeny. The majority of gene sequences found in the bacteriophage genetic repertoire are novel sequences with no known function (62). Because most of these genetic sequences are completely unexplored, speculation on functions is difficult. The transcription of *orf10* during lysogeny suggests that it plays a role in the establishment or maintenance of the lysogenic state of Tuc2009. However, supplying *orf10* on the constitutive lactococcal-promoter-containing multicopy vector pNZ44 (63) in *L. lactis* UC509.9 did not affect the efficiency of plaquing (EOP) or the frequency of lysogeny during infection with Tuc2009 (data not shown). Nevertheless, the suggestion that *orf10* is a "moron" gene, whereby expression of the gene confers an advantage on the host, cannot be ruled out. *orf10* seems to be conserved in some P335 phages, and homologs of this gene (98 to 99% sequence identity) are found in phage ul36 (64) and within prophages found in lactococcal genomes, such as *L. lactis* IL1403 prophages bIL285 and bIL286 (65) and the *L. lactis* MG1363 prophage MG-3 (66).

In conclusion, we have demonstrated a temporal transcriptional host response of *Lactococcus* to infection with different species of bacteriophage. This has revealed similarly low impacts on overall host transcription, with phage-specific transcriptional responses, suggesting that there is no common host strategy in response to infection with bacteriophages from different species.

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