Temperate bacteriophages and lysogeny in lactic acid bacteria

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1. SUMMARY

Lysogeny is widespread in the lactic acid bacteria. The majority of lysogens can be induced by UV irradiation or treatment with mitomycin C, but indicator strains which allow lytic growth of the induced phage are often not easy to identify. A few temperate phages have been shown to transduce chromosomal and/or plasmid markers. Information about the molecular biology of the temperate phages from lactic acid bacteria is sparse and needs significant supplementation in order that these potentially valuable phages might be utilized more efficiently as tools for improving existing starter strains in dairy fermentations.

2. INTRODUCTION

The first report confirming the isolation of lysogenic strains of lactic acid bacteria was that of Reiter [1] in 1949. He found that Lactococcus lactis ssp. cremoris HP was an indicator strain for phages induced from three different L. lactis strains, and drew attention to the need to avoid the use in fermentations of mixed starters containing both a lysogen and an indicator strain for the temperate phage.

Whilst the major attention of microbiologists in the dairy field has been focussed on the lytic phages, there has been an ongoing effort since Reiter’s discovery to learn more about temperate phages. In part this has been inspired by the possibility that virulent forms of temperate phages could have deleterious effects on dairy fermentations. The fact that temperate phages can be useful for transduction has also made them worth studying, especially since gene transfer systems for the lactic acid bacteria have been limited. An additional reason for investigating these phages is the prospect that they may be useful tools for introducing and stabilizing desirable genes in the bacterial chromosome. For these reasons and out of pure curiosity temperate phages have been studied and been observed to be present in many strains of the lactococci and the lactobacilli. As shall be seen, much of the analysis of temperate phages has been concerned with the identification of indicator strains and with the morphology of the phage particle. The use of a number of temperate phages to achieve transduction has been reported and details of the structure of the genome of some temperate phages have also been documented. However, there is a marked dearth of reported investigations into the molecular aspects of the life cycle of these organisms. The resulting paucity of information in this area is in sharp contrast to our detailed knowledge about tem-
perate phages in *Escherichia coli* and *Bacillus*, and must act as a barrier to the use of temperate phages as tools for improving the commercial properties of the lactic acid bacteria.

This article is devoted specifically to lysogeny and temperate phages in lactic acid bacteria. A number of earlier reviews include a discussion on these topics. In particular the papers by Lawrence et al. [2], Davies and Gasson [3], Teuber and Lembke [4], Klaenhammer [5] and Schaud et al. [6] are pertinent.

3. THE EXTENT OF LYSOGENY

3.1. *Lactococcus*

Reports in the literature reveal that more than 300 strains of *L. lactis* ssp. *lactis* and *cremoris* have been found to be lysogens [7–20]. The general conclusion that can be reached from these studies is that lysogeny is widespread in cheese starter strains and that multilysogenic strains also occur [15,21,22]. Whether all cheese starter strains are lysogens is not certain, since the failure to observe lysogeny by the methods in use does not in itself prove the absence of lysogeny.

In most studies starters have been treated with either UV light or mitomycin C to induce the prophage and then the turbidity of the culture has been monitored. The strain is generally regarded as lysogenic if the turbidity stops increasing after an additional 1–4 h incubation and then decreases, thereby indicating lysis. In more thorough investigations indicator strains on which the phage is capable of forming plaques have been sought. Huggins and Sandine [13] found that treatment of 63 strains with UV light or mitomycin C yielded intact phages or incomplete phage particles in 38 cases, even though indicator strains detected phage from only seven of the 63 strains. The presence of incomplete phage particles was also noted in other studies [14,16,20,22].

3.2. Curing and relysogeny

The observation that the treatment of a culture with either UV light or mitomycin C leads to cell lysis and the appearance of phage particles and plaque forming units is only a prima facie case for lysogeny. Formal proof of lysogeny requires that (a) the lysogen can be cured of its prophage; (b) the cured derivative is susceptible to infection by the temperate phage, and (c) the cured derivative can be relysogenized [23]. Gasson and Davies were the first to report this formal proof of lysogeny for a lactococcal strain [24]. They exposed single colonies of *L. lactis* ssp. *lactis* C2, *L. lactis* ssp. *lactis* 712 and *L. lactis* ssp. *cremoris* R1 to UV light in order to select spontaneously-cured derivatives. These derivatives did not lyse after UV treatment, thereby indicating the absence of functional prophage, yielded plaques when treated with lysates of their respective parental strain, thereby indicating susceptibility to reinfection, and yielded lysogens after this reinfection.

An alternative approach to using indicator strains to demonstrate the presence of phage in induced cultures has been to visualize them by electron microscopy [7,9,10,12–14,16]. By this method, Huggins and Sandine [13] found that treatment of 63 strains with UV light or mitomycin C yielded intact phages or incomplete phage particles in 38 cases, even though indicator strains detected phage from only seven of the 63 strains. The presence of incomplete phage particles was also noted in other studies [14,16,20,22].

More recently, the process of prophage curing and relysogeny of *L. lactis* ssp. *lactis* C2 derivatives has been confirmed by probing Southern blots of *EcoRI* digests of the chromosome with phage DNA [26]. This experiment therefore engenders not only logical satisfaction but demonstrates the
technical feasibility of curing commercial strains from their prophages.

The curing of *L. lactis* ssp. *cremoris* R1 had no effect on a range of metabolic activities, including rates of acid production and milk coagulation [25], or on sensitivity to three non-temperate phages [17]. Chopin et al. [15] studied prophage-cured derivatives of four lactococcal strains and reported that two of the cured strains showed altered sensitivity to infection by other phages. One of the cured strains gave slow acid production in milk, but this could not be unambiguously correlated with the loss of the prophage.

### 3.3. Lactobacillus

In general the study of phages and lysogeny in lactobacilli has lagged behind that in lactococci [6,27]. The field has been reviewed recently by Sechaud et al. [6]. Lysogeny in the genus *Lactobacillus* was first reported by Coetzee and de Klerk [28], who subjected 343 strains of *Lactobacillus* (from seven species) to UV irradiation and identified two lysogens. An additional 80 strains were examined in a later study in the same laboratory [29] and culture supernatants of 16 strains inhibited the growth of other strains. Phage-like particles were observed in eight of the 16 supernatants. Higher frequencies of lysogeny were observed when mitomycin C was employed for induction. Using this agent, 23 out of 30 *Lactobacillus salivarius* strains were found to be lysogenic by Tohyama et al. [30], and Yokokura et al. found 40 lysogens out of 148 *Lactobacillus* strains that included seven different species [31]. An even higher proportion of lysogens, 17 out of 21, was reported by Stetter [32], also using mitomycin C for induction. On the basis of these studies it can be concluded that lysogeny is common in *Lactobacillus*. The lysogen *Lactobacillus casei* S-1 is particularly interesting. A prophage was suspected to reside in this strain but could not be induced by treatment with either UV light or mitomycin C [33]. After mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, derivatives were obtained that had become thermosensitive, i.e., they could be induced by heat treatment. One of these mutants yielded cured derivatives which in turn could be re-lysogenized with the phage. Formal proof of lysogeny in *Lactobacillus* was thereby demonstrated for the first time. More recently, Raya et al. have unequivocally proven lysogeny in *L. acidophilus* ADH by the temperate phage Φadh [34].

### 3.4. Other lactic acid bacteria

Lysogeny of *Streptococcus salivarius* ssp. *thermophilus* has not yet been reported. Attempts to induce phages with mitomycin C or UV irradiation have been unsuccessful (H. Neve, unpublished observations). In an alternative approach, Neve and co-workers probed chromosomal DNA of seventeen *S. salivarius* ssp. *thermophilus* strains with the DNA of non-temperate phages and found that two strains hybridized strongly. Both of these strains were inducible with low concentrations of mitomycin C, releasing defective phage particles. Prophage-cured derivatives could be readily isolated and subsequently these derivatives could be re-lysogenized. Thus lysogeny has also been formally proven in this group of lactic acid bacteria, although it appears to be a rare event. The parental lysogens and the re-lysogenized derivatives grew homogeneously in broth, whereas the prophage-cured isolates aggregated and sedimented rapidly.

There appear to be no published reports on the existence of lysogeny in the genera *Pediococcus*, *Leuconostoc* or *Propionibacterium*.

### 4. PHAGE MORPHOLOGY, STRUCTURE AND TAXONOMY

#### 4.1. Morphology

As a consequence of the need to make extensive use of electron microscopy for monitoring prophage induction, the temperate phages of the lactic acid bacteria have been much photographed. With the exception of a small number of prolate-headed examples [15,30,35] all temperate phages from *Lactococcus* and *Lactobacillus* are isometric-headed [4,6,7,13,14,22,25,27,29,32,34–40]. Head diameters vary from 56 to 78 nm and tail lengths from 120 to 182 nm, except for some phages
which have longer tails (250–300 nm) [34,37,40]. Phages which are almost tail-less have been induced from L. lactis ssp. cremoris S18/4 and Lactobacillus salivarius [30,35]. Collars, base plates and tail fibres have been noted in various cases. In one study alone at least 20 ultrastructurally discernible temperate phages were observed [4].

4.2. Structure of the genome in the phage particle

By comparison with the body of information that has been built up about the morphology of these phages, our knowledge of their genomes is limited. A restriction map has been reported for only five temperate phages (Table 1). In addition, maps have recently been determined for the linear, 45-kbp genomes of two temperate phages from S. salivarius ssp. thermophilus (H. Nève, unpublished observations) and for phages mv1 and mv4 from Lactobacillus delbrueckii ssp. bulgaricus (M. Mata, unpublished observations). These maps and the combined estimate from electron microscopy data and fragment sizes for PL-1 DNA provide us with the most reliable information concerning genomic size (Table 1). Estimates of the genome size of some temperate phages have been obtained by summing up the sizes of fragments in individual DNA digests (Table 1). While this method provides useful interim values, it should be remembered that it can underestimate the total genome size.

The genome of the temperate lactococcal phage BK5-T, like that of ΦFSW, is circularly permutated. Electron microscopy revealed that BK5-T DNA consists of linear molecules, ranging in size from 39.7 to 46 kbp [41]. There is therefore considerable variability between the sizes of the DNA in individual BK5-T phage particles. Given a unit genome size of 37.6 kbp [41] measured by restriction mapping, the DNA molecules packaged in the BK5-T phage head must have terminal redundancies of between 2 and 8 kbp. Lakshmidevi et al. proposed that packaging of BK5-T DNA into the phage head occurs by a headful mechanism acting on a precursor concatameric molecule, and that this mechanism is not particularly precise with respect to DNA length [41]. The first step in packaging would involve cleavage of the concatamer at a specific site (referred to as pac), then a headful quantity of DNA would be packaged into a phage head. The location of pac on the restriction map and the direction of packaging of the DNA was determined [41].

Three genes have been localized on the restriction map of Lactobacillus phage mv1 and found to be transcribed in the same direction (M. Mata, unpublished observations). These are the genes for the two major phage proteins and the gene for lysin. The lysin gene has been cloned and expressed in E. coli, and its nucleotide sequence determined. The sequence data indicate that the lysin coding region, which is 586 bp long, is part of an operon.

Table 1
Details of temperate phage genomes

<table>
<thead>
<tr>
<th>Phage</th>
<th>Parent lysogen</th>
<th>Genome type</th>
<th>Genome size (kbp)</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK5-T</td>
<td>cremoris ² BK5</td>
<td>Linear, circularly permuted</td>
<td>37.6</td>
<td>RM</td>
<td>41</td>
</tr>
<tr>
<td>ÄT187, ÄT188, ÄT189</td>
<td>cremoris ² 187, 188, 189</td>
<td>ND</td>
<td>39–51</td>
<td>FS</td>
<td>42</td>
</tr>
<tr>
<td>PL-1</td>
<td>casei ³ ATCC27092</td>
<td>Linear, cohesive ends</td>
<td>38</td>
<td>FS, EM</td>
<td>40</td>
</tr>
<tr>
<td>ÄFSW</td>
<td>casei ³ S1</td>
<td>Linear, circularly permuted</td>
<td>41.5</td>
<td>RM</td>
<td>43</td>
</tr>
<tr>
<td>Äadh</td>
<td>acidophilus ³ ADH</td>
<td>Linear, cohesive ends</td>
<td>41.7</td>
<td>RM</td>
<td>34</td>
</tr>
<tr>
<td>mv4, mv1</td>
<td>bulgaricus ⁴ LT4, LT1</td>
<td>ND</td>
<td>38 ³</td>
<td>RM</td>
<td>44</td>
</tr>
<tr>
<td>Ä38</td>
<td>bulgaricus ⁴ B29</td>
<td>ND</td>
<td>33–37</td>
<td>FS</td>
<td>44</td>
</tr>
</tbody>
</table>

¹ Method for determining genome size: RM, restriction mapping; FS, fragment size; EM, electron microscopy.
² L. lactis ssp. cremoris.
³ Lactobacillus spp.
⁴ Lactobacillus delbrueckii ssp. bulgaricus.
⁵ M. Mata, unpublished observations.
4.3. Structure of the prophage genome

Hybridization analyses, using phage DNA to probe Southern blots of restriction digests of host chromosomal DNA, have been published for four lactococcal phages, \( \Phi T \) C2 (induced from \( L. \) lactis ssp. \( L. \) lactis LM0220), BK5-T, R1-T and 134-T [26,41,45] and two \( Lactobacillus \) phages, \( \Phi FSW \) and \( \Phi adh \) [34,43]. In each case the data are consistent with the hypothesis that the prophage consists of linear DNA integrated at a specific site in the bacterial chromosome. Additional, less favoured sites of integration may also exist in the chromosome [26,46]. The approximate location of the attachment site within the phage DNA has been determined for BK5-T, \( \Phi FSW \) and \( \Phi adh \) [34,41,43].

Mata and her colleagues have used pulsed field gel electrophoresis and labelled phage DNA to explore the location of mv4 prophage in the genomes of two strains of \( Lactobacillus \) delbrueckii ssp. bulgaricus, LKT and LT4 (M. Mata, unpublished observations). They found that mv4 was integrated into a single \( NotI \) restriction fragment (49 kbp) in strain LT4 and into two \( NotI \) fragments (23 and 18 kbp) in strain LKT. Surprisingly, an extrachromosomal linear form of mv4 was detected in both lysogens, more abundant than the integrated form.

4.4. Phage capsid proteins

Available information on the sizes of major proteins of temperate phages is summarized in Table 2. Of interest is the suggestion that the 23-kDa protein in \( \Phi T187 \) and relative compounds is a component of the phage tail [42]. On the basis of reported protein gel separations, it should not be difficult to purify sufficient quantities of these proteins to carry out amino acid sequence analysis for the identification of the coding region for the protein in the genome.

4.5. Phage taxonomy

There are insufficient data to attempt a definitive classification of these phages. Comprehensive studies comparing the genomes and structural proteins of many phages have not been carried out. However, the known properties of characterized phages indicate considerable diversity.

\( Lactobacillus \) phages PL-1 [40] and \( \Phi adh \) [34] are isometric-headed tailed phages, as is \( \Phi FSW \) [39], but differences between their genomic properties suggest that there are at least two distinct phage types. The varied morphologies of \( Lactobacillus \) temperate phages [6] indicate that several classes exist.

Similar diversity has been observed with lactococcal phages. Jarvis et al. [48] have proposed at least three characterized classes of temperate phages, but the relationships (if any) between these classes remain unclear.

5. VIRULENCE AND LOSS OF LYSOGENIC ABILITY

5.1. Virulence

Mutant temperate phages that have gained the ability to infect the parental lysogen are classically referred to as virulent phages. Genetic analysis of
the mutation(s) causing virulence is of particular interest since it can yield valuable information about regulation of the phage life cycle. As discussed below (Section 8) the development of virulence in temperate phages of commercial strains is also of commercial interest. The best documented example of virulence in the lactic acid bacteria is provided by the incisive investigations of Shimizu-Kadota and co-workers into the temperate phage OFSW (carried by *Lactobacillus casei* S-1) and its virulent derivatives OFSV-A, OFSV-B and OFSV-C [39,43,49].

These workers isolated OFSV from skim milk fermentations that employed *Lactobacillus casei* S-1 and observed that OFSV was capable of lytic infection of *Lactobacillus casei* S-1. In a series of experiments it was shown that *Lactobacillus casei* S-1 is lysogenic for a phage designated OFSW, and that three independent isolates of OFSV were indistinguishable from OFSW on the basis of morphology, serotype and the sizes of their structural proteins [33,39]. Further analysis revealed that the DNA of two of the isolates of OFSV, namely OFSV-B and OFSV-C, contained an additional 1.3 kbp of DNA compared with OFSW [39]. Subsequently the exciting discovery was made that the extra 1.3 kbp was a novel transposable element, named ISL1 [49]. Unfortunately for those interested in genetic regulation in phage, the Japanese group has concentrated on ISL1 and there have been no additional reports on the structures of the phage genes that were disrupted by the transposon insertion. Presumably, as the authors suggested, the insertion of ISL1 either destroyed the binding site of a phage regulatory protein or caused overexpression of a phage gene [49]. Further studies on this system would be most interesting.

5.2. Loss of lysogenic ability

In our laboratory we have observed that the temperate phage BK5-T, isolated from *L. lactis* ssp. *cremoris* BK5, can spontaneously lose the ability to form lysogens [46]. We found that *L. lactis* ssp. *cremoris* H2, an indicator strain for phage BK5-T [13,45], could be lysogenized with BK5-T provided that the sample of BK5-T was obtained by induction of a lysogen (either *L. lactis* ssp. *cremoris* BK5 or lysogenized *L. lactis* ssp. *cremoris* H2). However, when phage was prepared by lytic infection of *L. lactis* ssp. *cremoris* H2 (phage referred to as BK5-T.H2) the indicator strain could not be lysogenized. Further analysis revealed that the loss of ability to lysogenize was associated with a small deletion in the phage genome. Electron microscopy of heteroduplexes of DNA from BK5-T.H2 and BK5-T.BK5 (phage prepared by induction of *L. lactis* ssp. *cremoris* BK5) indicated that the size of the deletion varied from 0.6 to 2.5 kbp, with a mean value of 1.5 kbp (H. Neve, unpublished observations). The location of the deletion in the restriction map of BK5-T DNA was determined. It is quite separate from the phage *att* site. The nature of the gene(s) affected by the deletion are of interest, since it is possible that they encode functions affecting the establishment or maintenance of lysogeny, e.g. a repressor protein or an integrase.

6. TEMPERATE PHAGE LIFE CYCLE

Latent periods for the induction of temperate phages are usually between 1 and 4 h [8,9,19,26]. Shorter latent periods have been observed when the phage infects its indicator strain [8]. Lack of data on the molecular biology of the life cycle is one of the major deficiencies in our knowledge of temperate phage infecting lactic acid bacteria. Interesting data have been reported about the transcription of the DNA of *Lactobacillus casei* phage PL-1 [40]. PL-1 is a temperate phage that was initially thought to propagate only by a lytic cycle [32,50]. It has a narrow host range, probably because otherwise susceptible host strains are lysogens of homoimmune phages [32]. The PL-1 genome, which is a 38-kbp linear molecule bearing cohesive ends, is transcribed in vivo by the host RNA polymerase [40]. In vitro studies using highly purified host RNA polymerase showed that the entire phage genome was transcribed asymmetrically: 56% of the H strand and 43% of the C strand. The situation in vivo during a lytic cycle was also explored. The resulting data indicated an early-late regulation of transcription of PL-1 DNA in infected cells. That is to say, after the first 20
min of a 140-min latent period only 36% of the H strand had served as a template. By the 50 min mark all of the genome had been transcribed with a pattern of transcription identical to that observed in the in vitro studies. This work, like that described above on φFSW, has never been extended and it remains the only published account of investigations of the transcriptional events fol-
lowing the induction of, or the infection by, a lactic acid bacteria temperate phage.

We have carried out studies aimed at investigating genetic regulation in the lactococcal temperate phage BK5-T [51]. To locate phage promoters we used pMU1328, a promoter-detecting plasmid with E. coli and streptococcal replicons and a promoterless chloramphenicol acetyl transferase gene (cat) adjacent to multiple cloning sites [52]. Fragments of BK5-T DNA were ligated into pMU1328 and promoter-containing molecular clones were isolated after transformation of Streptococcus sanguis Challis lac8 to chloramphenicol resistance. The nucleotide sequence of five of the clones was determined along with the start point of transcription. The nucleotide sequence around the start point is shown in Fig. 1. The same start point was observed in both E. coli and S. sanguis, suggesting that the RNA polymerase in S. sanguis recognizes the same nucleotide sequences as the E. coli enzyme. Homology between the −35 and −10 regions of these promoters and the canonical E. coli promoter sequence is present (Fig. 1). To determine if any of these promoters was regulated by a BK5-T gene product, E. coli strains carrying

### Table 3

Effects of plasmids carrying BK5-T DNA on the activity of BK5-T promoters

<table>
<thead>
<tr>
<th>Promoter-containing plasmid</th>
<th>Promoter</th>
<th>Chloramphenicol acetyl transferase activity</th>
<th>Plasmid 2 co-resident with promoter-containing plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Promoter activity</td>
<td>pMU1252               pMU1253</td>
</tr>
<tr>
<td>In E. coli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pMU1261</td>
<td>Pf2</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>pMU1262</td>
<td>Pf1</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>pMU1265</td>
<td>Pg2</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>pMU1266</td>
<td>Pa1</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>pMU1268</td>
<td>Pa3</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>In L. lactis ssp. cremoris</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pMU1261</td>
<td>Pf2</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>pMU1262</td>
<td>Pf1</td>
<td>2.3</td>
<td>2.4</td>
</tr>
<tr>
<td>pMU1265</td>
<td>Pg2</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>pMU1266</td>
<td>Pa1</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>pMU1268</td>
<td>Pa3</td>
<td>2.4</td>
<td>2.5</td>
</tr>
</tbody>
</table>

1 Values are units of chloramphenicol acetyl transferase activity [56].
2 Plasmids contain EcoRI fragments of BK5-T DNA [41] as follows: pMU1252, EcoRI-d in pACYC184 [57]; pMU1253, EcoRI-f in pACYC184; pMU1271, EcoRI-f in pLS1 [58]; pMU1275, 864-bp EcoRI-HindIII fragment from EcoRI-f in pLS1.
Fig. 2. Nucleotide sequence of a BK5-T gene that reduces the activity of some cloned BK5-T promoters. The encoded amino acid sequence of the entire open reading frame is shown. Open circles below the nucleotide sequence indicate a potential ribosome binding site which would enable translation to commence at the 9th (methionine) codon.

each promoter clone were individually transformed with five different compatible plasmids carrying each of the five EcoRI fragments that cover the BK5-T genome [41]. Only the EcoRI-f fragment of BK5-T [41] caused any change, a reduction, in promoter activity as measured by chloramphenicol acetyl transferase levels (Table 3). Plasmids containing EcoRI-f were also introduced [59] into L. lactis ssp. cremoris H2 carrying the various promoter clones and were shown to have a similar effect on promoter activity to that observed in E. coli (Table 3). The region of the BK5-T fragment responsible for the reduction was localized by subcloning and Tn5 insertional mutagenesis to a stretch of DNA containing an open reading frame of 207 codons (Fig. 2). These results were interpreted as showing that the product of this open reading frame decreases the activity of some BK5-T promoters. Whether it is a repressor in the classical sense is not clear and requires further study.

7. TRANSDUCTION BY TEMPERATE PHAGE

Transduction is a valuable genetic tool which can be mediated by temperate phages. The early seminal experiments that discovered transduction of plasmids by the temperate phages of L. lactis ssp. lactis C2 [60] and L. lactis ssp. lactis 712 [37] have been reviewed [3]. The temperate phage of L. lactis ssp. cremoris C3 is also capable of transducing [36]. Evidence for transduction by phage BK5-T has also been obtained [46]. In this case transduction was investigated following the observation that restriction digests of phage DNA isolated after lytic growth on the indicator strain L. lactis ssp. cremoris H2 (BKS-T.H2, see section 5.2.) contained significant amounts of high-molecular weight DNA (approximately 40 kbp). The DNA in this band was heterogeneous in size and, on the basis of hybridization data, was found to be derived from the chromosome of L. lactis ssp. cremoris H2. Transduction of a chromosomal marker between derivatives of L. lactis ssp. cremoris H2 was observed at a frequency of $1.2 \times 10^{-8}$ per pfu. Plasmid DNA could also be transferred, albeit at the much lower frequency of $8.5 \times 10^{-9}$ per pfu. Southern blot analysis of the L. lactis ssp. cremoris H2 chromosome showed a number of sites on the chromosome that hybridized with the BKS-T EcoRI fragment containing the pac site. This suggests that there are pac-like regions in the bacterial chromosome and that these could initiate packaging of the chromosome into the phage head to produce transducing particles.

In Lactobacillus salivarius, generalized transduction of auxotrophic markers at frequencies of $10^{-8}$ to $10^{-7}$ per pfu was observed by Tohyama et al. [61]. Plasmid transduction has also been observed in this genus [34, 62]. Transduction of plasmids from Lactobacillus acidophilus ADH by its resident temperate phage 8adh occurred at frequencies of $8 \times 10^{-10}$ to $4 \times 10^{-8}$ per pfu, depending on the plasmid. This demonstration of transduction has obvious benefits in facilitating
the genetic characterization and manipulation of *Lactobacillus acidophilus* ADH. Equally important is the encouragement that this achievement will give to the search for transduction in other strains and species of this genus.

8. INDUSTRIAL SIGNIFICANCE OF LYSOGENY

As noted in the introduction, the possibility that temperate phages interfere with industrial fermentations has been a strong driving force behind the investigation of these organisms. Since the initial discovery of lysogeny in lactic acid bacteria, a range of conclusions has been expressed in the literature concerning the perceived magnitude of such interference [4, 5, 18, 22, 45, 47, 63].

One way in which a temperate phage could perturb fermentations would be by mutation to a virulent derivative, i.e. virulent in the classical sense of being able to overcome superinfection immunity. This type of mutation has the potential for causing a catastrophic failure in a lysogenic, single-strain starter culture, since the virulent phage could infect the entire starter population. The *Lactobacillus* phage OFSV which was isolated from abnormal fermentations in several factories [39] is an example of this process.

Another problem that could arise from using lysogenic starter strains is spontaneous induction giving rise to phage that infect a sensitive strain in a mixed starter culture [13, 64]. Clearly from the point of view of avoiding this situation it is unwise to use a mixed starter culture that contains a lysogen and its indicator strain; spontaneous induction is not impossible and has been observed in some cases [8, 10, 13, 18, 19]. The difficulty experienced in many laboratories in identifying indicator strains for temperate phages has led some workers to conclude that they are rare and that this situation arises infrequently in industry [4].

The close correlation between the lytic spectra of an extensive sample of temperate and non-temperate phages led Reyrolle et al. [18] to propose that temperate phages constitute a reservoir of phages that could mutate to a virulent form and cause problems in cheese making. This possibility has been explored by using DNA hybridization to measure the extent of homology between some temperate and non-temperate phages [42, 44, 45, 47, 65]. For lactococcal phages the overall conclusion so far from these experiments is that there is little or no homology between most temperate and most non-temperate phages. However, a few lytic phages, e.g. Φ49 and P335, showed considerable homology with some temperate phages [42, 65]. For *Lactobacillus* phages, homology has been observed between four temperate and 15 non-temperate phages [44]. The DNA of the two recently-discovered temperate phages of *S. salivarius* ssp. *thermophilus* has an extensive region of homology with the central 25% of the DNA of the non-temperate phage P55 (H. Neve, unpublished observations). It is clear therefore that at least some temperate and non-temperate phages share homology, suggesting that some non-temperate phages are variants of temperate phages, or that genes may have been exchanged by recombination during the evolution of these two phage types.

9. FUTURE DEVELOPMENTS

There are a number of ways in which temperate phages could be used in the future to benefit the dairy industry. These are: as vehicles for introducing genes into the chromosome in a stable form, as genetic tools via transduction, as a means of allowing the release of ripening and/or flavour-enhancing enzymes into the curd matrix [16, 66], and possibly as specialized cloning vectors for lactic acid bacteria.

From a reading of the preceding sections it will be clear that a major barrier to the development of temperate phages for these uses is our ignorance of their molecular biology. There is a need for transcript mapping to determine the location of phage promoters and the temporal order, if any, of their expression. Phage regulatory proteins must be identified and their target sites in the genome characterized. Hopefully, the current rapid advances in DNA sequencing technology that are inspired by an interest in other genomes [67] will, when combined with recent significant increases in funding for dairy research [68], have a major
impact on research into temperate phages. The determination of the complete nucleotide sequence of a temperate phage genome is now a realistic undertaking for a well-organized laboratory and would be a most significant advance in our knowledge. The data resulting from these three approaches would provide major insights into the nature of phage genes and their products, and the organization of the phage genome. These insights would greatly facilitate the use of these phages to improve existing starter strains.

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