

# Lysogeny in Lactic Streptococci Producing and Not Producing Nisin

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Eighty-seven strains of lactic streptococci (46 of *Streptococcus lactis*, 24 of *S. diacetilactis*, and 17 of *S. cremoris*) were tested for lysogeny; 12 *S. lactis* strains produced nisin. Lysogeny was found in five *S. lactis* strains (two of them were nisin producers) and in two *S. diacetilactis* strains. Four *S. lactis* and two *S. diacetilactis* lysogens liberated phages both spontaneously and after ultraviolet treatment, and one *S. lactis* strain liberated phages spontaneously only. No lysogens were found among the *S. cremoris* strains tested. An initial characterization of the lysogens and their phages was made. The lytic spectrum of some of the examined phages was very narrow (homospecific), whereas that of others was wide, including strains of the three investigated species.

Lysogeny has hitherto not been observed in group N streptococci even though bacteriophages have been isolated from these bacteria by many workers (4-10). The purpose of this work was to determine whether lysogeny occurs in lactic streptococci, including strains producing the antibiotic nisin, and to examine some properties of host-phage systems in these bacteria.

## MATERIALS AND METHODS

**Strains.** Eighty-seven strains were investigated, including 46 strains of *Streptococcus lactis*, 24 strains of *S. diacetilactis*, and 17 of *S. cremoris*; 12 of the *S. lactis* strains produced nisin. The strains were kept at 4 C and transferred once a week to a 10% water suspension of defatted powdered milk. The strains came from the Institute of Dairy Industry, Warsaw; Pure Dairy Cultures, Laboratory of Olsztyn; Department of Industrial Microbiology, Technical University of Łódź.

**Medium.** The medium consisted of the following: beef heart infusion, 1,000 ml; peptone (Gurr), 10 g; yeast extract (Difco), 10 g; NaCl, 5 g; glucose, 10 g; 1 M CaCl<sub>2</sub>, 1 ml; final pH of the medium, 7.2. The medium was autoclaved at 117 C for 15 min. This medium will be referred to as X.

**Equipment and chemicals.** The equipment and chemicals used were an ultraviolet (UV, bactericidal lamp (Westinghouse G 30 T8), Berkefeld N2 and Seitz EKX filters; and mitomycin C (Nutritional Biochemicals Corp.).

**Determination of the plaque-forming units.** Determination of the plaque-forming units was performed by the method of Horvath and Alföldi (4) or Gratia (see reference 1).

**Number of viable bacteria.** The number of viable bacteria (colony-forming units) was determined by the plate method. The strains were incubated in a water bath or incubator at 30 C.

## RESULTS AND DISCUSSION

**Search for lysogens.** Search for lysogens was performed by Fisk's method (2) looking for phages in bacterial culture filtrates. In the case of filtrates which hindered the growth of indicator strains, 10<sup>-1</sup> to 10<sup>-3</sup> dilutions of the filtrates were applied, as well as the undiluted filtrates. This was necessary to distinguish between inhibition of the growth of indicator strains caused by phages and that due to other factors, e.g., nisin, produced by lactic streptococci. This distinction is possible if the filtrates are diluted to 10<sup>-3</sup> since at this concentration only the presence of phages, but not the activity of other factors, is detected.

The culture filtrates were screened for phages after 3 and 18 h of incubation of the strains and after 3 h of incubation of strains which were treated with UV (usually 260 ergs per mm<sup>2</sup>) immediately prior to incubation.

Each strain was treated as a potential lysogen and as a potential indicator strain.

Five lysogenic strains were found among 46 *S. lactis* strains examined. Four of them (37, 40, 41, 45) liberated phages which could only multiply in one indicator strain, *S. lactis* 28. A fifth strain, 31, liberated phages which could multiply in several strains belonging to the

three examined species. Strains 40, 45, and 28 produced nisin.

Phages were obtained sporadically from five other *S. lactis* strains, but reproducible results were not obtained.

Two lysogenic *S. diacetilactis* strains (84 and 87) were found among the 24 investigated strains. Phages liberated by these strains were able to multiply in a number of strains from the three examined species.

No lysogens were found among the 17 tested strains of *S. cremoris*, in spite of the use of various experimental conditions.

**Spontaneous liberation of phages.** Eighteen-hour cultures were centrifuged and the cells were washed twice with Ringer's solution. The bacteria were then resuspended in a volume of Ringer's solution equal to the volume of the initial culture. The suspensions were diluted 1:100 with X medium. After taking samples to determine the number of infective centers (CI), the strains were incubated to examine the kinetics of the increase of the number of phages and living bacteria. The controls performed indicated that a maximum of 1 out of 500 observed plaques does not represent CI.

Table 1 contains data on the frequency of spontaneous induction which was 1 to 2% and 0.04 to 0.07% for *S. lactis* and *S. diacetilactis* strains, respectively.

**Effectiveness of induction depending on the dose of UV.** Bacteria from 18-h cultures were washed twice with Ringer's solution and then resuspended in the same solution; 2.5 ml of this suspension was poured onto each petri dish (5.5 cm in diameter) and irradiated with UV. The number of induced cells and of surviving bacteria were determined immediately.

A correlation between the number of induced

cells and the UV dose is presented in Table 1. At the optimal UV dose (260 ergs/mm<sup>2</sup>), 20 to 37% of the cells were induced in *S. lactis* and 7 to 10% were induced in *S. diacetilactis* strains.

Only in the case of *S. lactis* strain 31 was no increase in the number of induced cells after UV treatment found, as compared with spontaneous induction (data not presented in the table). Sometimes, a 90% decrease of the number of phages was observed in this strain after irradiation. In this strain, neither mitomycin C at various concentrations nor a raised temperature was effective in the induction of phages. Investigations now being performed suggest that in this strain the prophage is not integrated into the chromosome.

**One-step growth of phages.** Lysogen suspensions after UV irradiation (260 ergs/mm<sup>2</sup>) were diluted 1:100 with X medium and incubated. Samples were taken for determination of the plaque-forming units and the number of living bacteria. One-step growth of the same phages was determined in parallel after infecting sensitive bacteria. The results are presented in Table 2.

The course of one-step growth of all *S. lactis* phages was similar after induction. The latent periods were 80 to 100 min long, and the periods of growth 30 to 60 min; 40 to 46 phages were liberated from each cell. For both lysogens of *S. diacetilactis*, the latent period was 40 to 60 min, the period of growth 60 to 80 min, and 10 to 25 phages were liberated from each cell.

When sensitive bacteria were infected with these phages, the periods of one-step growth of the phages were shorter, but the yields of phages were similar to those obtained after UV induction of lysogens.

**Lysogenization of sensitive bacteria.** To check whether the isolated phages lysogenize indicator strains, suspensions of *S. lactis*

TABLE 1. Effectiveness of phage induction in lysogenic lactic streptococci by using various UV doses

UV dose (ergs/mm <sup>2</sup> )	No. of induced cells in strain (%)						Survival of bacteria (%)					
	<i>S. lactis</i>				<i>S. diacetilactis</i>		<i>S. lactis</i>				<i>S. diacetilactis</i>	
	37	40	41	45	84	87	37	40	41	45	84	87
0 (Spontaneous induction)	2	1	1	1	0.04	0.07	100	100	100	100	100	100
130	20	29	15	35	2.0	5	62	54	48	48	84	68
260	22	37	20	25	7.0	10	11	18	15	14	30	28
390	9	16	9	16	3.5	8	2	6	1	2	15	8
520	2	3	2	3	3	4	0.1	0.5	1	0.2	10	4
650	2	2	1	2	1	0.2	0.05	0.1	0.1	0.04	1	1

TABLE 2. One-step growth and burst size of UV-induced *S. lactis* and *S. diacetilactis* lysogenic phages<sup>a</sup>

Lysogen	Duration of latent period (min)	Duration of rise period (min)	Burst size
<i>S. lactis</i> 37	80-100 (60)	30-60 (30)	44 (40)
40	80-100 (60)	30-60 (30)	40 (40)
41	80-100 (60)	30-60 (30)	46 (40)
45	80-100 (60)	30-60 (30)	40 (40)
<i>S. diacetilactis</i> 84	40-60 (30)	60-80 (30)	25 (10)
87	40-60 (30)	60-80 (30)	10 (10)

<sup>a</sup> In parentheses, one-step growth and burst size of phages after infection of indicator strains *S. lactis* 28 and *S. diacetilactis* 86.

phages (with the exception of phages from strain 31) were applied to *S. lactis* strain 28, and those from *S. diacetilactis* strains of *S. diacetilactis* 86. All phages were able to lysogenize indicator strains, as most bacterial colonies which grew in the presence of the phages contained phage-resistant cells which liberate phages spontaneously and after UV induction.

**Delysogenization and relysogenization of nisin-producing strain *S. lactis* 45.** This strain was irradiated on solid medium with a UV dose of 650 ergs/mm<sup>2</sup> which inactivated 99.9% of the cells. Among the colonies grown after UV irradiation, five nonlysogenic ones were found which were susceptible to phage 45 and produced nisin. The subclones thus obtained were susceptible to relysogenization with phage 45.

**Lytic spectrum of the phages.** Phages at routine test dilution (RTD) and 1,000 × RTD concentrations were used for lytic spectrum determinations.

As is shown in Table 3, phages from strains *S. lactis* 37, 40, 41, and 45 were able to reproduce in *S. lactis* strain 28 only. The phages from *S. lactis* strain 31 and *S. diacetilactis* strains 84 and 87 had a wide lytic spectrum and were able to multiply in a number of strains (including four nisin producers) belonging to the same and to the two remaining species. The lytic spectrum was slightly more narrow when the phages were used at RTD concentration.

**Repressor resistance of *S. lactis* strain 28 after lysogenization by a single phage to the remaining *S. lactis* phages.** Investigations of the lytic spectrum and one-step growth of the isolated phages of *S. lactis* suggested a great similarity or even identity of these phages. To check whether these phages were identical, the

repressor resistance of *S. lactis* strain 28 (lysogenized with one of the phages) to the remaining phages was determined; it was performed by lysogenization of strain 28 with one of the four phages and by subsequent treatment of this strain with the three remaining phages at RTD concentrations.

The results presented in Table 4 suggest that phages 40, 41, and 45 produce a similar type of repressor, because cross-resistance to these phages is observed. It seems that this repressor differs from that produced by phage 37. This indicates that some subtle differences do exist between phage 37 and the remaining phages.

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TABLE 3. Lytic spectrum of phages isolated from lysogenic lactic streptococci<sup>a</sup>

Phages isolated from:	No. of sensitive strains					
	<i>S. lactis</i>		<i>S. diacetilactis</i>		<i>S. cremoris</i>	
	RTD	1,000 RTD	RTD	1,000 RTD	RTD	1,000 RTD
<i>S. lactis</i> 31	7	17	1	12	6	6
37	1	1				
40	1	1				
41	1	1				
45	1	1				
<i>S. diacetilactis</i> 84	11	12	8	17	3	3
87	8	12	8	17	3	3

<sup>a</sup> RTD, Routine test dilution.

TABLE 4. Repressor immunity of *S. lactis* strain 28 lysogenized with a single phage to other *S. lactis* temperate phages

Lysogen	Sensitivity to phage isolated from <i>S. lactis</i> strain no.: <sup>a</sup>			
	37	40	41	45
37, 28	-	-	-	-
40, 28	+	-	-	-
41, 28	+	-	-	-
45, 28	+	-	-	-

<sup>a</sup> -, Immune; +, sensitive.

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