Lysogeny in Lactobacilli

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(Received 20 February 1974; revised 13 May 1974)

SUMMARY

Strains of lactobacilli were examined for lysogeny after treatment with mitomycin C. Forty strains belonging to 7 species out of 148 strains of 15 species were lysed by mitomycin C. Lysis was strongly dependent upon the concentration of mitomycin C, temperature and the age of the cultures.

Phage-like particles were observed in 31 out of 40 lysates by electron microscopy. Particles from ten lysates – four from *Lactobacillus casei* B, five from *L. casei* C and one from *L. acidophilus* – produced plaques when plated on suitable indicator strains and were thus considered to be active phages. The remaining 21 lysates that contained phage-like particles failed to produce plaques but four of them inhibited the growth of other lactobacilli. It was concluded that the particles in these lysates were defective phages.

Activities, shapes, dimensions and serological properties of the newly induced phage-like particles were also studied.

INTRODUCTION

Coetzee & de Klerk (1962) reported that two strains of *Lactobacillus fermenti* out of 345 strains of lactobacilli were lysogenic upon u.v. irradiation. Recently, de Klerk & Hugo (1970) examined the induction of phage-like structures from *L. acidophilus* with exposure to mitomycin C (MC) and found that the culture supernatant fluids of 16 strains inhibited the growth of other strains of *L. acidophilus*.

In our Institute, investigations have been performed for several years on the induction of phages from lactobacilli by treatment with MC, and lysogeny in *L. casei* and *L. salivarius* has been reported (Sakurai, Takahashi & Arai, 1970; Sakurai, Tohyama & Arai, 1971; Tohyama, Sakurai, Arai & Oda, 1972). This paper describes our attempts to demonstrate lysogeny in other species of lactobacilli.

METHODS

Bacterial strains. The sources of the strains examined are listed in Table 1. Strains isolated in this Institute were identified according to the criteria proposed by Rogosa & Sharpe (1959). *Lactobacillus casei* was classified serologically into two groups, B and C, as described by Sharpe (1955).

Media. The media used were MRS medium (de Man, Rogosa & Sharpe, 1960), Rogosa's medium (Efthymiou & Hansen, 1962) and MRT medium (Murata, Soeda & Saruno, 1969). Each organism was cultured on the most appropriate medium for growth.

Induction of lysogenic lactobacilli with mitomycin C. Induction was carried out according to Otsuji, Sekiguchi, Iijima & Takagi (1959). MC was obtained from Kyowa Hakko Kogyo Co., Ltd, Tokyo, Japan. The organisms were cultivated in the presence of MC and turbidity

Table 1. *List of strains*

Species	Strain*	Source†
<i>L. helveticus</i>	NCIB8733, H5, H17, H19	A
<i>L. jugurti</i>	NCDO99, J2, J10, J12	A
<i>L. casei</i> B	YIT0001	
	NIRDH831, NIRDDECF, A121	A
	I138	B
	C201, C594, C782	C
<i>L. casei</i> C	NIRDRO94, ATCC7469	A
	I136, I337, I141, I142, I143, I11, I12, IAM1043, IAM1045, I17, I18, I110, I111, I113, I114, I118	B
	C47, C48, C182, C218, C232, C773, C775, C781, C783, 3793	C
<i>L. plantarum</i>	IAM1216	
	NIRDA164, NCIB6376, NCTC7220, P12	A
	ATCC8014, ATCC14917, I130	B
	No. 1 to No. 55	C
<i>L. brevis</i>	ATCC14869	
	NCIB8169, NCTC6107, X10, T6	A
<i>L. buchneri</i>	ATCC4005	
	NCIB8007	A
<i>L. bulgaricus</i>	B1, B8, B17, ATCC11842	A
<i>L. lactis</i>	L2, L10, L29, ATCC8001	A
<i>L. fermenti</i>	ATCC14931, ATCC14932	
	ATCC8289, ATCC9338	A
	LF3, LF8, LF8M, LF10, LF17	C
<i>L. acidophilus</i>	ATCC4356, ATCC4357, ATCC4963, ATCC9857, ATCC11506, ATCC19992, YIT0096, YIT0097	
	A1, A4, A11, A28	A
<i>L. cellobiosus</i>	ATCC11739	
<i>L. delbrueckii</i>	ATCC9649	
<i>L. jensenii</i>	ATCC25258	
<i>L. leichmannii</i>	ATCC4797	
<i>L. viridescens</i>	ATCC12706	

* NIRD: National Institute for Research in Dairying, Reading, Berkshire. NCDO: National Collection of Dairy Organisms, Reading. NCTC: National Collection of Type Cultures, Central Public Health Laboratory, London. NCIB: National Collection of Industrial Bacteria, Torry Research Station, Aberdeen. ATCC: American Type Culture Collection, Rockville, Maryland, U.S.A. IAM: Institute of Applied Microbiology, University of Tokyo, Japan. YIT: Yakult Institute for Microbiological Research, Tokyo, Japan.

† A: received from Dr Sharpe, National Institute for Research in Dairying, Reading. B: received from Dr Mitsuoka, Institute of Physical and Chemical Research, Saitama, Japan. C: isolated and maintained in this Institute.

was measured by a Klett-Summerson photoelectric colorimeter equipped with a No. 66 filter. Induction was considered to have occurred when the organisms lysed in the presence of MC.

Assay for activity of induced phage-like particles. After centrifugation at 10000 g for 20 min, culture supernatant fluids of those lactobacilli that lysed were spotted on a lawn of lactobacilli or were spread with an indicator in soft agar medium. Plates were incubated overnight and were observed for growth-inhibiting and plaque-forming abilities of induced phage-like particles.

Isolation of non-lysogenic strains. Non-lysogenic strains were isolated from lysogenic ones as described by Seaman, Tarmy & Marmur (1964) in *Bacillus subtilis*. They were used as indicator strains for assays of the activity of induced phage-like particles.

Preparation of anti-phage sera. Preparation of antisera and the assay for the neutralizing ability of the sera were performed as previously reported for phage PLS1, one of the *L. salivarius* temperate phages (Sakurai *et al.* 1971).

Electron microscopy. After cultivation with MC, the culture fluids of those strains that lysed were centrifuged at 10000g for 20 min. The supernatant fluids were ultracentrifuged at 80000g for 90 min in a Hitachi centrifuge (model 55P-2). The pellet was suspended in 0.1 M-ammonium acetate (pH 7.2), stained with 1% (w/v) phosphotungstic acid, and examined with a JEM-7A electron microscope.

RESULTS

Cell lysis in lactobacilli by MC

Forty strains belonging to 8 species, out of 148 strains of the 15 species tested, lysed in the presence of MC (Table 2).

Effect of the concentration of MC. Lysis was dependent upon the concentration of MC; 0.2 µg MC/ml was the minimum concentration for lysis in almost all the lactobacilli strains (Fig. 1a).

Fig. 1. (a) Effect of the concentration of MC on lysis in lactobacilli. *Lactobacillus plantarum* NIRD164 (—) and *L. casei* IAM1043 (---) were cultivated at 30 °C with various concentrations of MC. MC concn (µg/ml): ○, none; ●, 0.1; △, 0.2; ▲, 0.4; □, 0.8. (b) Effect of cultivation temperature on lysis. *Lactobacillus plantarum* NIRD164 (—) and *L. casei* IAM1043 (---) were cultivated with 0.4 µg MC/ml at various temperatures. Temperature (°C): ○, 28; ●, 30; △, 34; ▲, 37. (c) Effect of the age of cultures on lysis in *L. plantarum* NIRD164 by MC. At the times indicated by the arrows, MC was added to the culture medium at a final concentration of 0.4 µg/ml and the number of viable cells was assayed, followed by measurement of turbidity in the culture medium. MC concn (µg/ml): ○, none; ●, 0.4. Viable cell count: A, 1.8×10^7 /ml; B, 2.9×10^7 /ml; C, 2.9×10^8 /ml; D, 8.4×10^8 /ml.

Table 2. *Effect of mitomycin C on strains of lactobacilli*

Species	Conditions for MC induction		Strains lysed with MC	Phage-like particles present in lysate	Activity of phage-like particles	
	Medium	Temp. (°C)			Plaque formation	Growth inhibition
<i>L. helveticus</i>	MRS	37	NCIB8733	+	—	—
<i>L. casei</i> B	MRT	30	NIRDD ECF	+	+	+
			I138	+	+	+
			YIT0001	+	+	+
			C201	+	—	—
			C594	—	—	—
			C782	+	+	+
<i>L. casei</i> C	MRT	30	NIRDR094	+	+	+
			II 2	+	+	+
			IAM1043	+	+	+
			II 8	+	—	—
			II 13	+	—	—
			C47	+	+	+
			C48	+	—	—
			C182	+	—	—
			C781	+	—	—
			3793	+	+	+
<i>L. plantarum</i>	MRT	30	IAM1216	+	—	—
			NIRDA164	+	—	—
			No. 1	+	—	—
			No. 4	—	—	—
			No. 7	—	—	—
			No. 10	—	—	—
			No. 11	—	—	—
			No. 12	—	—	—
			No. 19	—	—	—
			No. 23	—	—	—
			No. 28	+	—	—
			No. 31	+	—	—
			No. 45	+	—	—
			No. 54	+	—	—
<i>L. brevis</i>	MRT	30	NCIB8169	+	—	+
			NCTC6107	+	—	+
			X10	+	—	+
<i>L. buchneri</i>	MRT	30	NCIB8007	+	—	+
<i>L. fermenti</i>	Rogosa's	37	LF8M	+	—	—
<i>L. acidophilus</i>	Rogosa's	37	ATCC4963	+	—	—
			ATCC11506	+	—	—
			ATCC19992	+	+	+
<i>L. cellobiosus</i>	Rogosa's	37	ATCC11739	—	—	—

Effect of the temperature. Strains were cultivated with 0.4 µg MC/ml at various temperatures. *Lactobacillus plantarum* NIRDA164 lysed weakly at 37 °C but *L. casei* IAM1043 lysed distinctly (Fig. 1*b*). Therefore, each strain has an optimum temperature for lysis.

Effect of the age of cultures. MC was added to the culture media of *L. plantarum* NIRDA164 at various culture ages. Lysis was maximum when MC was added during early log phase (Fig. 1*c*). This was observed in all the lactobacilli strains lysed in the presence of MC.

Fig. 2. Phage-like particles induced from *L. casei* B (C782).

Fig. 3. Phage-like particles induced from *L. casei* C (112).

Phage-like particles in lysates

Phage-like particles were observed with an electron microscope in 31 lysates (Table 2). Photographs and dimensions of phage-like particles induced from *L. casei* C782, *L. casei* 112, *L. plantarum* No. 31 and *L. brevis* NCTC6107 are shown in Figs. 2 to 5 and Table 3 respectively. Almost all the induced particles appeared to have polyhedral heads and long, sheathless tails. However, some had polyhedral heads and contractile tail sheaths or a tail-less head-like structure which was frequently seen to be empty. The shape and dimension of the particles were similar to those of temperate phages from *L. fermenti*, *L. acidophilus*, *L. casei* and

Fig. 4. Phage-like particles induced from *L. plantarum* No. 31.

Fig. 5. Phage-like particles induced from *L. brevis* NCTC6107.

L. salivarius reported previously (de Klerk, Coetzee & Fourie, 1965; de Klerk & Hugo, 1970; Sakurai *et al.* 1970, 1971; Tohyama *et al.* 1972).

Activity of the induced phage-like particles. Lysates containing phage-like particles were examined for their abilities to form plaques and inhibit growth when plated on suitable indicator strains. Particles from nine strains of *L. casei* and one strain of *L. acidophilus* showed both plaque-forming and growth-inhibiting abilities for strains of each species (Table 2). The non-lysogenic strains were good indicators for phage-like particles induced from *L. casei* c. All of the phages were species-specific in their range of activity, with the

Table 3. *Dimensions of phage-like particles induced from lactobacilli*

Values are the means of ten measurements.

Particles from	Head* (nm)	Tail length (nm)	Sheath width (nm)	Core width (nm)
<i>L. casei</i> C782	48	152	—	8
<i>L. casei</i> 112	62	182	—	5
<i>L. plantarum</i> No. 31	57	—	—	—
<i>L. brevis</i> NCTC6107	69	280	26	8.5

* Dimension from apex to tail joint.

Table 4. *Serological relationship between phages from L. casei B and C*

Phages were incubated with a serum at 37 °C for 90 min.

Values represent residual plaque-forming units after incubation with antisera.

Phages from	Antiserum against phages from		
	NIRDDECP	NIRDRO94	IAM1043
<i>L. casei</i> B			
NIRDDECP	1.4×10^{-3}	1.0	1.0
1138	3.5×10^{-3}	1.0	1.0
C782	5.8×10^{-3}	1.0	1.0
YIT0001	1.0	1.0	1.0
<i>L. casei</i> C			
NIRDRO94	1.0	1.9×10^{-3}	1.0
3793	1.0	2.6×10^{-3}	1.0
IAM1043	1.0	2.7×10^{-1}	1.0×10^{-2}
C47	1.0	2.1×10^{-1}	1.6×10^{-2}
112	1.0	1.0	1.0

possible exception of those released by *L. buchneri* NCIB8007 which inhibited the growth of a non-lysogenic derivative of *L. brevis* NCTC6107. The number of plaque-forming units varied in the different lysates from 10^2 to 10^8 per ml. Neither growth-inhibiting nor plaque-forming ability of the particles from *L. helveticus*, *L. plantarum* and *L. fermenti* has been observed.

Serological relationships between phages induced from L. casei B and C

Antisera against one *L. casei* B phage and two *L. casei* C phages were tested for their abilities to neutralize four *L. casei* B phages and five *L. casei* C phages (Table 4). There was no apparent serological relationship between the *L. casei* B and C phages. Three of the four *L. casei* B phages showed a serological relationship in that they were neutralized to more or less the same extent by serum produced against one of them. The fourth *L. casei* B phage, that released by strain YIT0001, was serologically distinct. The five phages induced from strains of *L. casei* C could be divided into three serological groups: those released by strains NIRDRO94 and 3793 belonged to one group, those released by strains IAM1043 and C47 to a second group, and those released by strain 112 to a third group.

DISCUSSION

There are only a few reports of lysogeny in lactobacilli. This may be due to a failure to provide suitable conditions for induction. Some conditions for the induction of phage-like particles were suggested for *L. salivarius* (Sakurai *et al.* 1971) and *L. casei* (Ishiwa & Sakurai, 1972). Our results show that induction of lactobacilli by MC is strongly dependent on the concentration of MC, temperature of incubation and the age of the culture at the time of exposure. Failure to detect lysogeny may also be explained by the fact that many strains appear to release defective phage particles. In the present work, the majority of phage-like structures induced by MC showed no activity against the indicator strains that were used. It should be noted that lysis induced by MC cannot be taken as evidence of lysogeny. Thus nine out of 40 strains that were lysed by MC did not release any phage-like structures detectable by electron microscopy of the concentrated lysates.

The authors wish to thank Mr S. Iwata for his technical assistance in the electron microscopic examinations.

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