Lysogeny in *Leuconostocs*

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Abstract Thirty-two leuconostoc strains were examined for lysogeny with mitomycin C. Bacterial lysis with induction of mitomycin C occurred in one strain out of seven *Leuconostoc cremoris* strains, one strain out of seventeen *Leuconostoc dextranicum* strains and three strains out of seven *Leuconostoc mesenteroides* strains. One *Leuconostoc lactis* strain was not inducible. Bacterial lysis with induction of mitomycin C in leuconostocs depended strongly on the concentration of mitomycin C in the culture. Phage-like particles in each of the lysates of *Leuconostoc mesenteroides* AHU 1068, AHU 1071 and *Leuconostoc dextranicum* IAM 1122, that were induced with mitomycin C, had the ability to form plaques.

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One of main factors causing slow acid development in the manufacture of fermented dairy products by lactic acid bacteria is due to virulent phage infection to the dairy starter. The control of phage infection to dairy starter that is widely used in the manufacture of fermented dairy products is an important problem. Several reviews¹⁻³⁾ have been reported on the virulent phage active against dairy lactic acid bacteria. Lysogeny has been also known to be widespread among lactic streptococci⁴⁻⁹⁾ and lactobacilli¹⁰⁻¹⁵⁾. Lysogenic strain in dairy starter may act as a reservoir of phage, and temperate phage⁶⁻⁸⁾, that is released from lysogenic strain of lactic acid bacteria used as dairy starter, may be able to infect other dairy lactic acid bacteria. This means that dairy starter may be infected by temperate phage as well as virulent phage. Although many reports presented on lysogeny of lactic streptococci and lactobacilli as indicated above, no paper has presented on lysogeny of leuconostoc species. In the previous paper^{16,17)}, the authors reported the isolation and the morphology of leuconostoc phages and phage-like particles induced from leuconostoc strains with mitomycin C. The authors examined for lysogeny of thirty-two strains from stock culture of leuconostoc species in authors laboratory and found that several leuconostoc strains were induced by mitomycin C.

This paper describes the results of our attempts to demonstrate lysogeny in leuconostoc species.

Materials and Methods

Leuconostoc strains

The origin of all the leuconostoc strains used in this study has been already reported¹⁶⁾.

Medium and growth of leuconostoc strains

Lactic broth¹⁸⁾ supplemented with 0.15% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was used for growth and induction of leuconostoc strains used in this study. Each of the leuconostoc strains was grown in lactic broth at 27°C by 2% inoculum of 18 hours culture into lactic broth. After incubation for 18 hours each strain was stored at 4°C.

Induction of lysogenic leuconostoc strain with mitomycin C

0.1 ml of an 18 hours culture of each of the leuconostoc strains was inoculated into 5 ml of lactic broth and then the inoculated broth was incubated at 27°C. When optical density of the culture at 660 nm in a Bausch and Lomb Spectronic 20 colorimeter reached to approximate 0.1, mitomycin C (Kyowa Hakko Kogyo Co., Ltd, Tokyo Japan) was added to the culture at a final concentration of 0.5 $\mu\text{g/ml}$ or 5.0 $\mu\text{g/ml}$. The culture was incubated at 27°C until bacterial lysis through induction with mitomycin C occurred.

Assay of lytic activity of phage-like particles in the lysate induced from leuconostoc strains with mitomycin C

Lytic activity of phage-like particles was determined by plaque formation using lactic agar (1.5% agar) and top layer (0.4% agar) according to the double layer method of ADAMS¹⁹⁾. That is to say, 0.1 ml of the lysate of leuconostoc strain induced with mitomycin C was plated on a lactic agar with each of the several leuconostoc strains out of thirty-two leuconostoc strains used in this study. The plate was incubated at 27°C overnight and then was observed for plaque forming ability of phage-like particles.

Results and Discussion

Induction of leuconostoc species by mitomycin C

Effect of mitomycin C concentration on typical induction of leuconostoc species is shown in Fig. 1 and Fig. 2. Although *Leuconostoc dextranicum* IAM 1122 was induced by a range of mitomycin C concentrations from 0.3 to 1.0 $\mu\text{g/ml}$ in the culture, it was not inducible above mitomycin C concentration of 3.0 $\mu\text{g/ml}$ in the culture. *Leuconostoc cremoris* IAM 1215 was induced by a range of mitomycin C concentrations from 3.0 to 5.0 $\mu\text{g/ml}$ in the culture, but was not inducible below mitomycin C concentration of 1.0 $\mu\text{g/ml}$ in the culture. These results indicated that induction of each of the leuconostoc strains depended strongly on the concentration of mitomycin C. Induction of lactobacillus strains¹⁴⁾ was carried out by a range of mitomycin C concentrations of 0.1 to 0.8 $\mu\text{g/ml}$. Induction of lactic streptococci was carried out by a range of mitomycin C concentrations of 0.5 to 2.0 $\mu\text{g/ml}$ ⁶⁾, 0.1 to 5.0 $\mu\text{g/ml}$ ⁷⁾, 1.0 to 5.0 $\mu\text{g/ml}$ ⁸⁾ and 0.4 to 6.0 $\mu\text{g/ml}$ ⁹⁾.

These ranges indicated that the strain-specific differences are in responses to mitomycin C concentration for induction of lysogenic strain. Thirty-two leuconostoc strains were examined for lysogeny with mitomycin C by a range of its concentrations of 0.5 μg to 5.0 $\mu\text{g/ml}$ in the culture. The results of induction of leuconostoc strains

Fig. 1. Induction of bacterial lysis in *Leuconostoc dextranicum* IAM 1122 by mitomycin C. At the time indicated by the arrow, mitomycin C was added to the culture at various concentrations: ($\mu\text{g/ml}$): 0 (○-○), 0.3 (●-●), 0.5 (▲-▲), 1.0 (■-■), 3.0 (●-●), 5.0 (▲-▲).

with mitomycin C are shown Table 1. One strain out of seven *Leuconostoc cremoris* strains, one strain out of seventeen *Leuconostoc dextranicum* strains and three strains out of seven *Leuconostoc mesenteroides* strains were induced with mitomycin C. *Leuconostoc lactis* ATCC 19256 strain was not inducible.

Lytic activity of phage-like particles

In the previous paper¹⁷⁾, the authors reported that the morphology of the phage-like particles was observed in each of the lysates induced from *Leuconostoc cremoris* IAM

Fig. 2. Induction of bacterial lysis in *Leuconostoc cremoris* IAM 1215 by mitomycin C. At the time indicated by the arrow, mitomycin C was added to the culture at various concentrations: ($\mu\text{g/ml}$): 0 (○-○), 0.3 (●-●), 0.5 (▲-▲), 1.0 (■-■), 3.0 (◐-◐), 5.0 (◑-◑).

1215, *Leuconostoc mesenteroides* AHU 1068, AHU 1071 and *Leuconostoc dextranicum* IAM 1122, but was not observed in the lysate induced from *Leuconostoc mesenteroides* AHU 1072. The lytic activity of these phage-like particles was examined according to the double layer method of ADAMS. The results are shown in Table 2.

The phage-like particles in the lysate induced from *Leuconostoc dextranicum* IAM

Table 1. Bacterial lysis of leuconostocs by induction of mitomycin C.

Leuconostoc strains*		Bacterial lysis**
<i>Leuconostoc cremoris</i>	ATCC 19254	—
"	IAM 1215	+
"	NO 42-13	—
"	NO 44-1	—
"	NO 44-2	—
"	NO 44-3	—
"	NO 44-4	—
<i>Leuconostoc lactis</i>	ATCC 19256	—
<i>Leuconostoc dextranicum</i>	ATCC 19255	—
"	IAM 1122	+
"	AHU 1076	—
"	AHU 1077	—
"	AHU 1078	—
"	AHU 1080	—
"	NO 2-2	—
"	NO 2-4	—
"	NO 2-6	—
"	NO 11-8	—
"	NO 21-7	—
"	NO 21-8	—
"	NO 42-1	—
"	NO 46-4-4	—
"	NO 46-6	—
"	NO 46-8	—
"	NO 47-6	—
<i>Leuconostoc mesenteroides</i>	ATCC 10830	—
"	IAM 1046	—
"	IAM 1647	—
"	AHU 1068	+
"	AHU 1071	+
"	AHU 1072	+
"	AHU 1073	—

*ATCC: American Type Culture Collection, Rockville, Maryland, U.S.A. IAM: Institute of Applied Microbiology, The University of Tokyo, Japan. AHU: Faculty of Agriculture, Hokkaido University, Japan. NO: These groups were isolated, and maintained in author's laboratory. **Positive indicates a significant decrease of optical density at 660 nm of culture after addition of mitomycin C (0.5~5.0 $\mu\text{g/ml}$) to culture.

1122 with mitomycin C have the ability to form plaques on lawns of *Leuconostoc mesenteroides* AHU 1068 and AHU 1073. The phage-like particles in the lysate from *Leuconostoc mesenteroides* AHU 1068 have the ability to form plaques on lawn of *Leuconostoc dextranicum* IAM 1122. The phage-like particles in the lysate induced from *Leuconostoc mesenteroides* AHU 1071 have the ability to form plaques on lawns of

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Table 2. Lytic activity of phage-like particles in lysate of *Leuconostoc* strain induced with mitomycin C.

			Plaque formation			
			1215-lysate ^{a)}	1122-lysate ^{b)}	1068-lysate ^{c)}	1071-lysate ^{d)}
<i>Leuc. mesenteroides</i>	IAM	1046	—	—	—	—
"	IAM	1067	—	—	—	—
"	AHU	1068	—	+	—	+
"	AHU	1071	—	—	—	—
"	AHU	1072	—	—	—	—
"	AHU	1073	—	+	—	+
<i>Leuc. dextranicum</i>	IAM	1122	—	—	+	+
"	AHU	1076	—	—	—	—
"	AHU	1077	—	—	—	—
"	AHU	1078	—	—	—	—
<i>Leuc. cremoris</i>	IAM	1215	—	—	—	—

Lytic activity was assayed in each of the lysates of *Leuconostoc cremoris* IAM 1215^{a)}, *Leuconostoc dextranicum* IAM 1122^{b)}, *Leuconostoc mesenteroides* AHU 1068^{c)}, and *Leuconostoc mesenteroides* AHU 1071^{d)} induced with mitomycin C. + indicates plaque formation, and — indicates no plaque formation on a plate seeded with each of the host bacteria. *Leuc*: *Leuconostoc*

Leuconostoc mesenteroides AHU 1068, AHU 1073 and *Leuconostoc dextranicum* IAM 1122. Although leuconostoc phages¹⁶⁾, that were isolated from dairy products, have very narrow host ranges, the phage-like particles had the broad ability to form plaques (Table 2). There were considerable doubt as to whether *Leuconostoc dextranicum* IAM 1122, *Leuconostoc mesenteroides* AHU 1068, AHU 1071 and AHU 1073 had physiological properties as *Leuconostoc dextranicum* species or *Leuconostoc mesenteroides* species, and the authors admitted that it is necessary to re-identify these strains.

The re-identification of these four strains were carried out and then were classified into *Leuconostoc dextranicum* species depending on the properties of dextran production, production of aroma compounds, production of acid from arabinose, etc., according to the method of the authors²⁰⁾.

From the results of identification of above these leuconostoc strains, it has become apparent that phage-like particles induced from leuconostoc strains with mitomycin C have narrow lytic patterns to leuconostoc species. Details on the physiological characterization of these strains will be reported by the authors in near future.

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Leuconostoc 菌種の溶原性

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Lactic streptococci および lactobacilli の溶原性についていくつかの報告が行われているが, leuconostoc 菌種の溶原性についての報告は, 著者らが知るかぎりにおいて見い出せない. 本報告は, leuconostoc 菌種の溶原性の検索を目的とし leuconostoc 32 菌株をマイトマイシン C 処理により, その溶原性の有無を調べた. Leuconostoc 菌株の溶原性のマイトマイシン C による誘発は, その濃度に大きく依存したマイトマイシン C に対する感受性は, leuconostoc 菌株によってこととなった. Leuconostoc 菌株を培養し対数期初期にマイトマイシン C を

液体培地 1 ml 当り最終濃度 0.5 μ g および 5.0 μ g 加えて溶原菌株の誘発を行った. *Leuconostoc cremoris* 7 菌株中 1 菌株に, *Leuconostoc dextranicum* 17 菌株中 1 菌株に, および *Leuconostoc mesenteroides* 7 菌株中 3 菌株にマイトマイシン C 処理により誘発が起り溶菌した. この 5 溶菌液中 4 溶菌液に phage-like particles の存在をすでに電子顕微鏡によって確認・報告したが, この 4 溶菌液中 3 溶菌液はプラーク形成能を有していた.

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