Bacteriophages in fermented meat products

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Summary

During a commercial dry sausage (salami) fermentation, a virulent bacteriophage Φ LP 65 was isolated which lysed the *Lactobacillus plantarum* LP 65 starter culture. Further characterization revealed a close morphological relationship to Φ fri, a phage which also lyses the starter strain. However, the two phages were different in several other characteristics. Furthermore, a temperate phage of *L. plantarum* LP 65 could be induced by mitomycin C. No similarities with the virulent phages were found. The possibility, that the low competivity of the starter strain may be due to phage infection or spontaneous induction of the temperate phage, is discussed.

Introduction

Dry sausages are frequently produced by the use of starter cultures. Lactic acid bacteria are used because of the transformation of sugars into lactic acid, which leads to a drop in pH. Sausages produced with *Lactobacillus plantarum* usually acidify faster than spontaneously fermented sausages. However, the starter strain is often overgrown by spontaneous lactic acid bacteria. This may be due to a low competivity of *L. plantarum*. Another possibility for this phenomenon may be the action of bacteriophages which lyse the starter cultures. Phages have sporadically been isolated from commercial meat starter cultures or from dry sausages (Gyllenberg & Hackman 1961, Biewald 1961, Sozzi *et al.* 1973, Trevors *et al.* 1983, Götz *et al.* 1984, Marchesini *et al.* 1991), but only two reports investigated the possible influence of phages on the fermentation (Trevors *et al.* 1984, Nes & Sorheim 1984). The results were contradictory, thus there is no clear evidence for an influence of phages on the fermentation.

Materials and Methods

Bacterial Strains and Phages

The bacterial strains and phages used in this study are listed in Table 1.

Phage Isolation

Homogenates of sausage samples were supplemented with 1/10 volume of MRS medium and 1/100 volume of a culture of *L. plantarum* LP 65 and incubated at 30 °C for 16 h. The supernatant was sterile filtered and the filtrate was checked for the presence of phage with a broth culture of strain LP 65.

Induction by Mitomycin C

At various stages of growth, 1 µg/ml mitomycin C was added to the cultures and growth was followed by measuring optical density. Cleared lysates were sterile filtered and phages shown by electron microscopy.

Isolation and Purification of Phage

Phages were collected from lysates by two consecutive centrifugations at 12,000 *g* (30 min) and 26,000 *g* (2 h). The pellet was redissolved in STM buffer (50 mM Tris-HCl pH 8.0, 10 mM NaCl, 10 mM $MgCl_2$) and the phages purified in a CsCl gradient.

SDS-PAGE Electrophoresis

Polyacrylamide gel electrophoresis of purified phages was carried out with 12 % SDS-PAGE gels in a BioRad Mini-Protean II electrophoresis device.

Preparation of Phage DNA and Restriction Analysis

To the purified phage suspension, 20 mM EDTA, 100 µg Proteinase K and 0.5 % SDS were added, incubated at 37 °C for 2 h and after several precipitations with phenol, the aqueous supernatant which contained phage DNA was precipitated with ethanol. Restriction enzymes from Boehringer Mannheim were used according to the suppliers recommendations.

Results

Isolation of a Virulent Phage

From a commercial dry sausage fermentation, a phage could be isolated which lysed the starter organism *L. plantarum* LP 65 (Lamirlac). The phage Φ LP 65 was isolated between day 1 and day 8 of the fermentation.

Isolation of Temperate Phages

L. plantarum LP 57 (Duploferment) and LP 65 (Lamirlac) were tested for lysogeny by induction with mitomycin C. Lysis could be induced, but the presence of phage could only be shown by electron microscopy, as no indicator strains for the released phages could be found. Characterization of the two phages Φ LP 571 and Φ LP 651 showed that they were identical.

Characterisation of Phages.

The main characteristics of the phages are compiled in Table 2. The lysis patterns of Φ fri and Φ LP 65 are given in Table 1. Electron microscopy showed a close relationship between the two virulent phages, whereas the temperate phage was morphologically different. Lysis patterns, SDS-PAGE and restriction endonuclease patterns of the phage DNA showed that the two virulent phages were different, whereas the temperate phages were identical in all characteristics. This confirmed our assumption, that the two *L. plantarum* strains LP 57 and LP 65 were identical.

Discussion

Starter organisms used for meat fermentations are susceptible to lysis by bacteriophage. We have previously isolated phages lysing *Staphylococcus carnosus* from fermenting sausages in Germany and Italy. During a commercial fermentation of Salchichon in Spain, we isolated phages lysing the *L. plantarum* starter strain. Starter organisms are meant to represent the main flora during the fermentation. For *L. plantarum* this is not always the case. After 1 to 2 days of fermentation, *L. plantarum* is often overgrown by the spontaneous flora which mainly consists of *L. sake* or *L. curvatus*. When the cell counts are determined on MRS agar plates, this succession in population

is not obvious unless the colonies are identified separately. Nevertheless, *L. plantarum* seems to favour growth of the spontaneous flora which grew slower when no starter was used.

Why do the numbers of *L. plantarum* decrease soon after addition to the sausage mix? Among other possibilities, phage attack has never been seriously considered. There are only two reports about experiments where the influence of phages on starter cultures was investigated. Whereas Trevors *et al.* 1984 found no influence on the fermentation, Nes & Sorheim 1984 could show, that the starter was diminished when phages were inoculated together with the starter. In fermented milk products diffusion is easy and phage propagation is fast whereas in meat products the solid matrix hinders free dispersal of phage.

Temperate phages from starter organisms can be induced by mitomycin C. This induction may also occur spontaneously, which would be a potential drawback of the use of lysogenic starter cultures. This has not yet been considered in the development of starter cultures.

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Table 1

Bacterial Strains, Phages, and Host-Specificity				
STRAINS/PHAGES	TAXONOMY	SOURCE	SENSITIVITY Φ fri	SENSITIVITY Φ LP 65
LP 57	*L. plantarum*	Duploferment, Müller, Gießen, Germany	+	+
LP 65	*L. plantarum*	Lamirlac, Lamirsa, Barcelona, Spain	+	+
DSM 20174	*L. plantarum (T)*	Deutsche Sammlung von Mikroorganismen	-	+
DSM 20205	*L. plantarum*	Deutsche Sammlung von Mikroorganismen	-	-
DSM 2601	*L. plantarum*	Deutsche Sammlung von Mikroorganismen	-	+
DSM 20246	*L. plantarum*	Deutsche Sammlung von Mikroorganismen	-	-
ATCC 8014	*L. plantarum*	American Type Culture Collection	-	-
54 Natural isolates	*Lactobacillus spec.*	Natural Flora Salchichon, Spain	-54/+0	-38/+16
Φ fri		Virulent phage of *L. plantarum* LP 57		
Φ LP 65		Virulent phage of *L. plantarum* LP 65		
Φ LP 651		Temperate phage of *L. plantarum* LP 65		

T Type strain

Table 2

Morphological Characteristics			
	Φ fri	Φ LP 65	Φ LP 651
HEAD	isometric hexagonal		
Ø nm	102 ± 2	100 ± 4	63 ± 2
TAIL	contractile		non contractile
Length nm	186 ± 9	177 ± 7	259 ± 9
Width nm	19	19	9
BASEPLATE	+	+	+