Staphylococcus carnosus bacteriophages isolated from salami factories in Germany and Italy

Barbara Marchesini, Anne Bruttin, R. Moreton and T. Sozzi
Nestlé Research Centre, Vers-chez-les-Blanc, Lausanne, Switzerland

Summary

Two phages lysing strains of *Staphylococcus carnosus*, an organism used as a starter culture for salami production, have been isolated from factories in Germany and Italy. Morphologically they show the C1 morphotype and are unrelated to the only other known *S.carnosus* phage. The phages were physiologically and morphologically similar but showed differences in their structural proteins and DNA restriction patterns. Their genomes consisted of linear double stranded DNA with a genome size of 19 kb. The phages lysed a wide range of *S.carnosus* strains from commercial meat starter cultures as well as the DSM type strain. Despite the presence of these phages, the products were normal from the point of view of colour, texture and flavour.

Introduction

Starter cultures are added to fermented meat products to increase the acidification rate and improve the colour, texture and flavour of such products. The most common organism used for colour stabilisation and flavour production is *Staphylococcus carnosus*, whereas Lactobacilli or Pediococci are used for acidification. In countries where sodium nitrate is added to the curing salts, a strong nitrate reducer such as *S.carnosus* is important to reduce nitrate to nitrite which reacts with the muscle myoglobin to give the pink colour, nitrosyl myoglobin, associated with these products. Use of starter cultures produces more consistent, reproducible products than those made by natural fermentations. Where nitrate reduction is not important, in countries such as Germany which use nitrite cures, *S.carnosus* still appears to have a positive effect on colour and flavour and is used for this purpose. In dairy fermentations for the production of cheese and yoghurt, bacteriophage infections cause major problems and serious economic loss due to failed fermentations. In most cases these problems can be controlled by use of rotations of phage unrelated strains or use of phage resistant strains. With fermented meat products, a similar situation would seem to be possible although there has been relatively little work in this area. Nes and Sørheim (1984) showed with a *L.plantarum* phage that presence of this phage could delay acidification when the homologous *L.plantarum* was used as starter. However, Trevors *et al* (1984), using a different *L.plantarum* phage, concluded that such problems were of no industrial significance. There have been no previous reports of *S.carnosus* phages isolated from fermented meat products. During a study of commercial meat fermentations in two unrelated salami factories in Germany and Italy, we have isolated two similar but not identical *S.carnosus* phages of a type different from the only other reported *S.carnosus*phage. These phages lysed a wide range of *S.carnosus* strains isolated from commercial meat starters, but not *S.aureus* or Micrococcus strains.

Results

By electron microscopy, both phages show the relatively rare C1 morphotype characterised by a hexagonal isometric head approximately 48 nm in diameter and a short non-contractile tail 27 nm in length. Between the head and the tail is a circular collar structure 47 nm in diameter with 12 appendages which appear to terminate in either spherical or hook-like structures. The tail appears to terminate in a spherical or hemispherical structure with a narrow region between the cylindrical tail section and the terminal portion. There is no base plate or tail fibres. The latent period of infection was about 40 min. for each phage followed by a rise time of 10 min and a burst size of about 60. ϕ stc 1 (Germany) was 90% adsorbed in 5 min compared to 58% with ϕ stc 2 (Italy).

After 15 min both phages were 98-99% adsorbed. Phage structural proteins showed 7 bands with ϕ stc 1 with calculated molecular weights of 80, 62.5, 53, 44, 39 and 34 kDa. An additional band with a molecular weight of 29.8 kDa was seen with ϕ stc 2. The phage DNA was resistant to restriction by the endonucleases Dpn I, Hind III, BamH I, Kpn I, Xho I and Nhe I but was cut by Bgl II and Pvu I (ϕ stc 1). There were additional sites for Bcl I and EcoR I and a second Pvu I site on the ϕ stc 2 DNA. Restriction maps were constructed from these fragments. Phage DNA was also cut by Sau3A I and Mbo I producing too many fragments to use in the restriction map.

The genome size of both phage DNAs was estimated by addition of the sizes of these fragments to be 19 kb. The genome is linear double stranded DNA. The DNA base composition was determined by HPLC after nuclease P1 and phosphatase treatment. The G+C content of ϕ stc 1 DNA was 31.5 %, and ϕ stc 2 35.9 %. That of *S.carnosus* DSM 20501, the type strain, was 35.4 %. There was no evidence from the HPLC chromatograms of any modified bases. The literature value for *S.carnosus* DNA (Schleifer, 1986) is 35-36%. Both phages lysed the type strain of *S.carnosus* and 6/7 other *S.carnosus* strains (Table 1), but no other strains tested of *S.xylosus* or *Micrococcus varians*, also used as meat starters, or one wild isolate of *S.aureus*. ϕ sk 311 lysed all of the *S.carnosus* strains and 2/3 *S.xylosus* strains. The Staphylococcus phage ϕ mc 11, only lysed its homologous host. Plaques of both phages on lawns of host cells incorporated in solid N medium were of irregular size.

Discussion

In 1961, Gyllenberg and Hackman reported the isolation of a phage lysing an organism described as *Staphylococcus lactis* used in the manufacture of German sausage which came from a lysed starter culture provided by R. Müller & Co, Germany. Götz *et al*, (1984), isolated ϕ sk 311, also from a lysed starter culture, this time *S.carnosus*. The phages described in this report appear to be the first *S.carnosus* phages isolated from a fermented meat product, although they were obviously not very abundant since an enrichment technique was required to isolate them. The isolate of Gyllenberg and Hackman appears to be a group B phage, as is that of Götz *et al*. Phages stc 1 and 2 belong to the less common group C1 (Ackermann *et al*, 1984). The host specificity of phages stc 1 and 2 appears to be restricted to *S.carnosus* whereas ϕ sk 311 has a broad specificity lysing at least 8 coagulase negative Staphylococci commonly found in meat products as well as *S.aureus* (Götz *et al*., 1984). The latent period and rise time of the two phages was within the range normally found, but the burst size (~60) was lower than the normal range which may be relevant to the apparently low infectivity. The products from which these phages were isolated were apparently normal from the point of view of colour, texture and taste and phages were only isolated from relatively few samples, there certainly did not seem to be massive phage proliferation during the fermentation and ripening process. One reason for this could be that the added *S.carnosus* starters show relatively limited growth in these products. In the Italian salami, counts of Staphylococci increased from log 7.2/g on the day of production to log 7.9/g after 7 days and then fell. In Germany, from an initially low level of log 4.1/g on the day of production, Staphylococci increased to log 6.2/g after 1 day and then declined slowly (Marchesini *et al*., 1991). Bacteria are normally more susceptible to phage lysis during periods of active growth, whereas in these products the potential hosts grow either slowly or are in stationary phase and presumably are less susceptible to phage infections. Also, in comparison with the situation found in dairy fermentations where the growth medium is a well mixed liquid, in meat fermentations the substrate is a non-mixed solid which limits the possibility of the rapid propagation of phage infections. In the production situation these phages do not seem to present a serious problem, but their presence should be borne in mind when preparing starter cultures or in case of production problems.

References

. Götz, F., Popp, F. & Schleifer, K.H. (1984) Isolation and characterization of a virulent bacteriophage from *Staphylococcus carnosus*. *FEMS Microbiology Letters* 23, 303-307.

. Gyllenberg, H.G. & Hackman, C.R. (1961) A Bacteriophage-lysing strain of Staphylococcus employed in the manufacture of dry sausage. *Applied Microbiology* 9, 480-483.

. Marchesini, B., Bruttin, A., Romailler, N., Moreton, R., Stucchi, C. & Sozzi, T. (1991) Microbiological events during commercial meat fermentations. *Journal of Applied Bacteriology* (1991) submitted for publication.

. Nes, I.F., & Sørheim, O. (1984) Effect of infection of a bacteriophage in a starter culture during the production of Salami Dry Sausage : a model study. *Journal of Food Science* 49, 337-340.

. Schleifer, K.H. (1986) Gram positive cocci., In *Bergey's Manual of Systematic Bacteriology*, Vol. 2, ed. Sneath, P.H.A., Mair, N.S., Sharpe, M.E. & Holt, J.G. pp 1030-1031, Baltimore, Williams & Wilkins.

. Sozzi, T., Maret, R. & Cerise, L. (1973) Isolation and some characteristics of two Micrococcus phages from Italian salami, type Varzi. *Archives of Microbiology* 92, 313-320.

. Trevors, K.E., Holley, R.A. & Kempton, A.G. (1983) Isolation and characterisation of a *Lactobacillus plantarum* bacteriophage isolated from a meat starter culture. *Journal of Applied Bacteriology* 54, 281-288.

. Ackermann, H.W., Cantor, E.D., Jarvis, A.W., Lembke, J. & Mayo, J.A. (1984) New species definitions in phages of Gram-positive cocci. *Intervirology* 22, 181-190.

Table 1

HOST SPECIFICITY OF PHAGES AND STAPHYLOCOCCI/MICROCOCCI

STRAINS	TAXONOMY	SOURCE	SENSITIVITY			
			ϕ stc 1	ϕ stc 2	ϕ sk 311	ϕ mc 11
DSM 20501	Staph. carnosus	Deutsche Sammlung von Mikroorganismen	+	+	+	-
STC 1	" "	Duploferment/Müller (Germany)	+	+	+	-
STC 2	" "	Microstart/Hansen (Italy)	+	+	+	-
STC 3	" "	Floracarn/Hansen (Sweden)	+	+	+	-
STC 4	" "	Saga/Microlife (Germany)	+	+	+	-
STC 5	" "	Actif M/Schneider (Switzerland)	+	+	+	-
STC 8	" "	Bitek/Gewürzmüller (Germany)	-	-	+	-
TM 300	" "	Prof. F. Götz/Tübingen (Germany)	+	+	+	-
DSM 20266	Staph. xylosus	Deutsche Sammlung von Mikroorganismen	-	-	-	-
STX 1	" "	Biostart/Raps (Germany)	-	-	+	-
STX 1	" "	Biostart/Raps (Germany)	-	-	+	-
DSM 20033	M. varians	Deutsche Sammlung von Mikroorganismen	-	-	-	-
MCV 1	" "	Saga/Microlife (Germany)	-	-	-	-
MCV 2	" "	Saga/Microlife (Germany)	-	-	-	+
MC 11	Staph. sp.	Salami "Varzi"/Natural Flora (Italy) (Sozzi et al., 1973)	-	-	-	

Legend: + lysis
- no lysis