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Original article

Characterization of a phage infecting *Propionibacterium freudenreichii*

M Gautier, A Rouault, S Lortal, Y Leloir, D Patureau

INRA, Laboratoire de Recherches de Technologie Laitière, 65, Rue de St Brieuc, 35042 Rennes, France

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Summary — Bacteriophage B22 was isolated from a Swiss-type cheese. Out of 30 strains of *Propionibacterium freudenreichii*, only the strain TL110 was sensitive to this phage. B22 morphology is very similar to those of phages infecting *P acnes*. It has an isometric head, a non-contractile tail and a tail plate, so it belongs to the B1 group of Bradley's classification. The B22 genome consists of a linear double-stranded DNA molecule 40 kb long with cohesive ends.

bacteriophage / *Propionibacterium freudenreichii* / cheese

Résumé — Caractérisation d'un bactériophage infectant *Propionibacterium freudenreichii.* *Le bactériophage B22 a été isolé à partir d'un fromage à pâte pressée cuite. Il n'infecte qu'une souche (TL110) sur les 30 souches de* Propionibacterium freudenreichii *testées. Morphologiquement, B22 ressemble beaucoup aux phages infectant* P acnes. *Sa tête est un icosahèdre, sa queue est non contractile et il possède une plaque basale. Il se trouve donc dans le groupe B1 de la classification de Bradley. Son génome est constitué d'une molécule d'ADN double brin de 40 kilobases possédant des extrémités cohésives.*

bactériophage* / Propionibacterium freudenreichii / *fromage

INTRODUCTION

Propionibacteria are divided in 2 major groups: the dairy and the cutaneous species (Sneath *et al*, 1986). The main cutaneous propionibacteria species, *P acnes*, is well known because it is involved in the development of acne vulgaris. Many phages typing *P acnes* have been isolated and characterized (Zierdst, 1974; Webster and Cummins, 1978). Among the dairy propionibacterium species, *P freudenreichii* is widely used in Swiss-type cheese manufacture because of its ability to produce the typical flavor and eye opening (Langsrud and Reinbold, 1973a,b). To our knowledge, no phage active against the dairy propionibacteria has been detected although the infection of starters is a common feature of dairy processes. The present paper describes the first isolation and characterization of a phage active on a strain of *P freudenreichii.*

MATERIALS AND METHODS

Bacterial strains and culture conditions

The strains used in this study came from the ATCC (American Type Culture Collection), CNRZ (Centre National de Recherches Zootechniques, INRA, France), DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) and TL (INRA, Collection du Laboratoire de Recherches de Technologie Laitière, France). Strains were grown anaerobically at 30 °C in Yel medium (Hettinga *et al*, 1968) in jars. The propagation strain, TL110, was isolated in a Swiss-type cheese.

Phage detection

Fifty g of 7 various Swiss-type cheeses bought in cheese shops (Emmental) were mixed with an Ultraturrax (20 000 rpm) for 2 min in 60 ml of Yel medium. The samples were mixed and centrifuged for 20 min at 3 000 *g*. The supernatant was tested against 30 strains of *P freudenreichii* for bacteriophage detection using the soft agar layer method of Adams (1959). Phages were also checked in whey samples collected during Emmental cheese manufacture.

Phage titer was first approximately determined by spotting dilutions on Yela plates over laid with *P freudenreichii* TL110 culture. Then all the phages were assayed by the soft agar layer method of Adams using Yel medium. High-titer lysates were prepared by infecting an early-log-phase TL110 culture with B22 phage at a multiplicity infection of 0.1. The infected culture was then incubated at 30 °C until complete lysis which generally occurred after 48 h.

Phage purification

The clear lysate was centrifuged at 5 000 *g* for 20 min to remove cell debris, and phages were concentrated by precipitation with 10% polyethylene glycol 6 000 in the presence of 0.5 mol/l NaCl (Yamamoto and Alberts, 1970). Phage particles were then purified by banding on CsCl gradients as described by Maniatis *et al* (1982).

Electron microscopy

Concentrated suspensions of phages were stained as described by Accolas and Spillmann (1979) with uranyl acetate 5UA (2% w/v, pH 4.5). Stained grids were observed with a Zeiss EM-10 electron microscope operating at 80 kV.

SDS–polyacrylamide gel electrophoresis

Polyacrylamide gels consisted of a stacking gel (4% acrylamide, 0.3% bisacrylamide) and a running gel (15% acrylamide, 0.4% bisacrylamide) which were prepared according to Laemmli (1970). Purified and concentrated phage suspension (100 µl, 10^{10}–10^{11} PFU ml) was dried in a speed vacuum centrifuge (Savant Instruments). Thirty µl of SDS 5% and 20 µl of 0.05 mol/l Tris-buffer (pH 7.2) were added to the dried pellet. The samples were denatured for 10 min at 100 °C, and 70 µl of denatured solution (0.1% SDS, 0.1% mercaptoethanol, 30% glycerol, 0.2% orange G; all percentages in w/v in 0.01 mol/l Tris-HCl buffer, pH 7.2) were finally added. The gel was loaded with 10–50 µl per well according to the desired coloration. Pharmacia molecular mass markers (14–94 kDa) were used and staining was carried out with Coomassie brillant blue R250. Part of the gel was also stained using silver nitrate (Hancock and Poxton, 1988).

DNA enzyme digests

The digestion of phage DNA with restriction endonucleases was performed according to the manufacturer's instructions (Boehringer). The restriction DNA fragments were analyzed on agarose gels.

RESULTS AND DISCUSSION

Phage isolation

No phage was detected in the whey samples collected during cheese manufacture

but 4 phages were isolated from the Emmental cheeses. We decided to study the phage B22 active on the strain TL110 because the plaques were clear and without a halo, with a diameter of 1 mm and therefore easy to take off and propagate.

Morphology

Phage B22 displayed an isometric head and a non-contractile tail with a terminal plate (fig 1). The head measurements were 48.5–50 nm from base to apex and 50–53 nm in width. The average length of the tail was *ca* 120 nm and the width *ca* 6 nm. A tail plate was observed on few particles. These observations are very similar to those made by Zierdt (1974) with the *P acnes* phages. The morphology of these phages is also similar to those of some phages infecting *L lactis* (Bauer *et al*, 1970) and *Corynebacterium lilium* (Trautwetter *et al*, 1987). Thus phage B22 could be considered as belonging to Bradley's classification B1 group (1967).

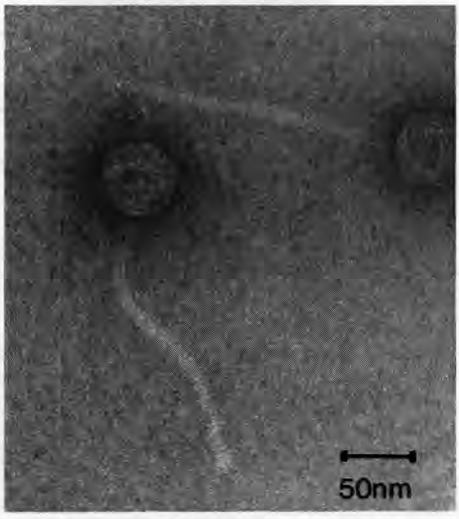

Fig 1. Electron micrograph of B22 phage stained with 2% uranyl acetate.
Photographie en microscopie électronique du phage B22 contrasté à l'acétate d'uranyle 2%.

Host spectrum

In order to overcome possible resistance mechanisms, we used a high concentration of phage (10^{11} PFU/ml) for typing. Phage B22 was assayed on 30 strains of *P freudenreichii*, 5 strains of *P jensenii*, 7 strains of *P acidipropionici* and 2 strains of *P thoenii*. No strain of *P thoenii*, *P jensenii* and *P acidipropionici* was infected with phage B22 according to the usual specificity of phage infection. Only the *P freudenreichii* TL110 strain was sensitive to this phage. Insensitivity of other *P freudenreichii* strains may be due either to the presence of a resistance mechanism or to the absence of a suitable adsorption site.

B22 phage structural polypeptides

SDS-PAGE analysis of the polypeptides from CsCl-purified B22 is shown in figure 2. Coomassie blue staining revealed only 3 proteins, with an estimated molecular weight of 35.0, 39.0 and 94.0 kDa; 2 additional minor proteins, of *ca* 15 and 25 kDa, were detected using silver staining. The profile was not similar to those obtained from phages infecting corynebacteria (Trautwetter *et al*, 1987).

DNA restriction pattern

B22 nucleic acid was a double-stranded DNA molecule since it could be digested by restriction endonucleases (Wells and Neuendorf, 1981). The genome size was 40 kb as determined by summing the size of the restriction fragments produced by each enzyme. B22 DNA digests were heated at 78 °C for 15 min, cooled rapidly on

ice, and loaded on agarose gels. For each digest, heating resulted in the disappearance of one restriction fragment and the appearance of 2 new fragments (fig 3). These results suggest the presence of cohesive ends. Indeed, circular DNA molecules which spontaneously formed by these sticky ends are known as unstable and are opened by heating.

CONCLUSION

To our knowledge this is the first description of a phage active on *Propionibacterium freudenreichii*. The phage in question belongs to Bradley's classification B1 group. The host spectrum, structural polypeptides and the DNA restriction pattern constitute a preliminary characterization which is soon to be completed by adsorption kinetics, one-step growth curve and chromosome restriction map. Further work implies determining the occurrence of bacteriophages in fresh curd as well as their possible effect on the ripening of Swiss type cheese.

In addition, since no functional cloning vector is available for the dairy propionibacteria, phage B22 DNA could be used to

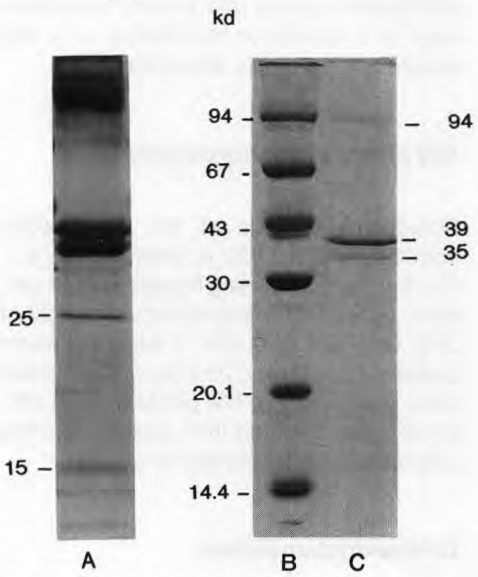

Fig 2. Pattern of CsCl purified B22 phage structural proteins obtained by SDS–PAGE. Lane A : silver nitrate staining. Lane B : molecular mass standards (Pharmacia). Lane C : Coomassie blue staining.
Profil des protéines du phage B22 purifié en gradient de CsCl obtenu en SDS–PAGE. Piste A : coloration au nitrate d'argent. Piste B : marqueurs de poids moléculaires (Pharmacia). Piste C : coloration au bleu de Coomassie.

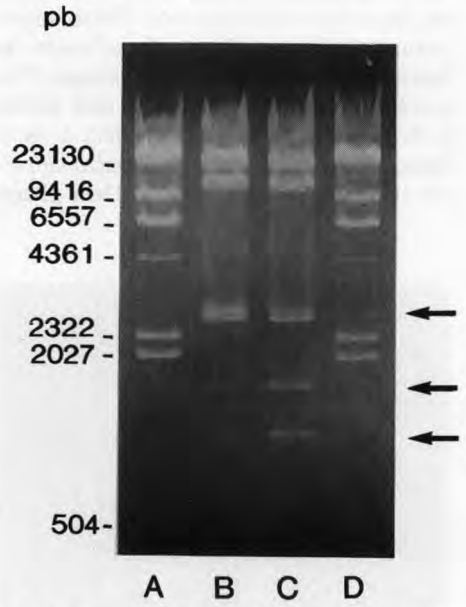

Fig 3. Observation of cohesive ends of B22 phage. Lanes A and D : Lambda DNA digested with *Hind* III and *Eco* RI. Lane B : phage B22 DNA digested with *Hind* III. Lane C : phage B22 DNA digested with *Hind* III and heated at 78 °C for 15 min.
Mise en évidence des extrémités cohésives du génome du phage B22. Pistes A et D : ADN du phage lambda digéré avec Hind *III and* Eco *RI. Piste B : ADN du phage B22 digéré avec* Hind *III. Piste C : ADN du phage B22 digéré avec* Hind *III puis chauffé à 78 °C pendant 15 min.*

optimize the techniques of DNA transfer in order to improve the technological properties of the strains.

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FUNCTIONS OF FERMENTED MILK

CHALLENGES FOR THE HEALTH SCIENCES

edited by **Y. Nakazawa, A. Hosono** *and*
Translated by **B.W. Howells**

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This book, the translation of a book originally written in Japanese, presents the current knowledge regarding the properties and roles of fermented milks and lactic drinks, and introduces methodologies and possibilities which could be applied within the health services. It is the result of a cooperative effort amongst researchers in various fields. Many topics of interest to health professionals are discussed, for example, the pathology of microorganisms in the body; the nutritional and physiological value of fermented milk products, and their potential as dietetic and prophylactic foods, and for prolonging life and maintaining health. The authors are specialists in a variety of fields encompassing medicine, veterinary medicine, hygiene sciences, food engineering, nutrition, immunology and microbiology.

In the first section, aspects of health are dealt with from the viewpoint of pathology. This section looks at the role of the human digestive tract and its association with intestinal microorganisms, and their influence on infectious disease, cancer and mutagenicity, adult diseases and ageing. The second section looks at fermented milks from a historical perspective and discusses the benefits of the many different types. Their nutritional and physiological value, and their potential as dietetic and prophylactic foods is considered. Topics such as longevity and food, cancer control, and milk products of long–lived countries are considered in the third section in the light of previous chapters on health aspects; and the implications of current progress in genetic engineering for the health sciences in the 21st century are also discussed.

This book should be read by all those with an interest in fermented milks and health: dairy scientists and technologists, nutritionists and dieticians, doctors and all those working within the dairy industry.

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