Analysis of the Collar-Whisker Structure of Temperate Lactococcal Bacteriophage TP901-1

Christina S. Vegge,† Horst Neve, Lone Brøndsted, Knut J. Heller, and Finn K. Vogensen

Department of Food Science, The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark; Institute for Microbiology, Federal Research Centre for Nutrition and Food, Kiel, Germany; and Department of Veterinary Pathobiology, The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark

Received 4 May 2006/Accepted 19 July 2006

Proteins homologous to the protein NPS (neck passage structure) are widespread among lactococcal phages. We investigated the hypothesis that NPS is involved in the infection of phage TP901-1 by analysis of an NPS− mutant. NPS was determined to form a collar-whisker complex but was shown to be nonessential for infection, phage assembly, and stability.

Bacteriophages of lactic acid bacteria (LAB) are an economic problem in the dairy industry, as phage infections can lead to slow or failed milk fermentation. Temperate Lactococcus lactis phage TP901-1 belongs to the P335 species of the Siphoviridae family, which is characterized by a small isometric head and a long noncontractile tail (1, 3, 18). The phage contains a collar and whiskers, which previous antibody-gold labeling experiments suggested were formed by the protein NPS (neck passage structure) (20), but the function of these structures is unknown. Recently, the collar-whisker labeling was confirmed for the similar lactococcal phage Tuc2009 (26). Collars and/or whiskers have often been observed on LAB phages (16, 17, 19, 21, 23), but these structures have not been further investigated. The corresponding complex of Escherichia coli phage T4 is formed from protein Gpwaec, which forms both the fiber-shaped whiskers and the ring structure attached to the phage neck (8, 9). The function of the T4 whiskers is to assist long tail fiber assembly and retraction (7, 30). However, only a single, relatively short tail fiber is found in TP901-1 (32), and it is therefore unlikely that the TP901-1 whiskers have a function equivalent to that of the whiskers of T4. Proteins homologous to NPS are encoded by more than 20 sequenced LAB phage genomes, and many of these proteins have been proposed to constitute host-interacting proteins (5, 11, 12, 29). In order to establish whether NPS does constitute the collar-whisker complex of TP901-1 and to explore the function of these structures, we have mutated nps of TP901-1 and analyzed the resulting mutant with respect to morphology, protein profile, infection efficiency, and stability.

A TP901-1 NPS+ prophage mutant was constructed by altering codons 76 and 77 of the nps gene (3) from ATA TCC to ATC TAG AAG, hence introducing an in-frame amber mutation and an XbaI site. This was accomplished using the pGhost8 vector as previously described (25, 32). NPS− phage were subsequently induced with mitomycin C, purified by isopycnic gradient centrifugation, and compared to the wild type (wt) phage and the previously described tailless TP901-1 Dit− mutant (32).

The morphology of the collar and whiskers was investigated by transmission electron microscopy (TEM) as described previously (32). The diameter of the collar of the wt phage was determined to be 16 ± 2 nm (n = 17), and at least two whiskers were found attached to the collar. The thin whiskers had a total length of 33 ± 4 nm (n = 44) and were found to carry small globular ends (Fig. 1A). The latter is a novel feature observed for whiskers of phages. The NPS− mutant was found to lack both collar and whiskers, while all other structures of this mutant were morphologically indistinguishable from those of the wt phage (Fig. 1B). Collar and whiskers were, however, found at the DNA-filled heads of the tailless Dit− mutant (Fig. 1C), showing that the collar-whisker complex actually is assembled on the head structure. This is in contrast to the whiskers of E. coli phage T4, which are assembled on the virion after the joining of head and tail (9).

The protein contents of the wt, NPS−, and Dit− phages were examined in 10% Novex bis-Tris polyacrylamide gels (Invitrogen) under reducing conditions (Fig. 2). This showed the NPS− mutant to be lacking a single band with the 72-kDa predicted size for the NPS protein. In view of the results of the morphological analysis, it was therefore concluded that the NPS protein forms the collar-whisker complex of TP901-1. However, it cannot be ruled out that additional minor structural proteins also may be present in the complex. The protein profiles furthermore confirmed that NPS is not required for assembly of the other structural components, as no additional protein bands were missing from the NPS− mutant, which supports a similar observation made for a comparable mutant of lactococcal phage bIL41 (10). In agreement with the TEM analysis, the 72-kDa NPS protein band was found to be present in the Dit− mutant, which therefore confirmed that NPS must be regarded as a head-associated protein. This result was unexpected, since nps is located downstream of the tail genes and 11 kbp away from the major head protein gene (3). Moreover, the NPS-like protein of lactococcal phage bIL170 was recently found to be lacking in a head-enriched fraction of bIL170 (10). However, the bIL170 heads examined were separated from...
tails by heat treatment in acidic, EDTA-containing buffer (10), and we therefore speculate that the NPS-like protein was lost during this stringent procedure.

The exact number of whiskers could not be determined by examining negatively stained phages with TEM, because this procedure can visualize structures only in one plane. However, we speculate that TP901-1 contains three whiskers, each formed by NPS trimers. This hypothesis is based on the facts that (i) no more than two whiskers were observed on wt TP901-1 and the tailless Dit\(^+\) mutant, (ii) the general symmetry of the portal vertex is 12-fold (4, 24), (iii) the NPS copy number is roughly estimated from the protein profile to be nine (results not shown), and (iv) the T4 whiskers and phage fibers in general appear to be trimeric (27).

In order to explore the possible host-interacting function of the NPS protein, several aspects of the infection process were examined and compared for the NPS\(^-\)/H11002 mutant and the wt phage. The infection efficiency of induced phage lysates was tested in plaque assays on host strain \textit{L. lactis} 3107 essentially as described by Lillehaug (22). These experiments revealed NPS\(^-\) mutant and wt phages to produce identical plaques (1 mm and turbid) at approximately the same titer (4 \times 10^9 PFU/ml [NPS\(^-\)] and 2 \times 10^9 PFU/ml [wt]). The infectious phage of the NPS\(^-\) lysate were subsequently verified as actual nps mutants by amplifying and sequencing the mutated regions from 10 plaque-forming isolates. All sequenced phages contained the amber mutation (results not shown), hence demonstrating that NPS is indeed not required for TP901-1 infection of \textit{L. lactis} 3107. To determine whether the NPS protein played a role in host range extension, as previously hypothesized for a homologous protein (11), the infectivity of the NPS\(^-\) lysate was tested in plaque assays on the two known TP901-1 indicator strains \textit{L. lactis} 3107 and Wg2 (6). However, no differences in host range or infectivity were observed, as identical titers were
TABLE 1. Rate of TP901-1 adsorption to L. lactis 3107

<table>
<thead>
<tr>
<th></th>
<th>Adsorption (%) at indicated min*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>wt</td>
<td>77</td>
</tr>
<tr>
<td>NPS mutant</td>
<td>84</td>
</tr>
</tbody>
</table>

* Percentage of adsorbed phages at different time points after mixing of phages and bacteria at an MOI of 0.1.

This work was supported by The Royal Veterinary and Agricultural University of Denmark. We thank Bernd Fahrenholz (FRCN Kiel) for assistance with the TEM.

REFERENCES


