ΦF1 and ΦF3, two novel virulent, archaeal phages infecting different thermophilic strains of the genus *Methanobacterium*

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Two virulent, archaeal phages, ΦF1 and ΦF3, were isolated that were capable of infecting different thermophilic members of the genus *Methanobacterium*. Both phages exhibited a similar morphology consisting of a polyhedral head and a tail but differed considerably in their host specificities and the size and topology of their genomes. Phage ΦF1 contained a linear, double-stranded DNA genome of 85 ± 5 kb in size and showed a broad host range including *M. thermoformicum* strains Z-245, FTF, FF1, FF3 and CSM3, and *M. thermoautotrophicum* strain ΔH. In contrast, ΦF3 phage particles contained a circular or terminally redundant linear genome, comprising approximately 36 ± 2 kb double-stranded DNA, and could only be propagated on *M. thermoformicum* strain FF3. Hybridization experiments did not reveal similarity between the genomes of ΦF1 and ΦF3 nor between both phages and genomic DNA from different thermophilic members of the genus *Methanobacterium* or DNA from phage ΨM1 of *M. thermoautotrophicum* Marburg. A physical map of both phage genomes was constructed. The DNA of phage ΦF1 was found to contain multiple GGCC sites which form the target for the restriction–modification (R/M) system *Mth*TI of *M. thermoformicum* THF. In contrast, the DNA of ΦF1 contained only a single CTAG site recognized by the R/M systems *Mth*ZI and *Mth*FI of *M. thermoformicum* Z-245 and FTF, respectively. The distribution of these sites correlates well with the capacity of ΦF1 to infect *M. thermoformicum* strains Z-245 and FTF but not strain THF.

Introduction

A variety of viruses and virus-like particles have been found in representatives of the three major lines of the archaeobacteria (now termed Archaea; Woese *et al.*, 1990), the methanogenic, halophilic and extreme thermophilic, sulphur-dependent organisms (for review, see Zillig *et al.*, 1988). All archaeal viruses and virus-like particles characterized so far contain double-stranded DNA genomes with sizes ranging from less than 30 kb for those infecting extreme thermophilic, sulphur-dependent Archaea up to 230 kb for the phage Jai of *Halobacterium halobium* (Zillig *et al.*, 1988; Wais *et al.*, 1975).

For methanogens, at least one virus-like particle, isolated from *Methanococcus voltae* A3 (Wood *et al.*, 1989) and a putative prophage of *Methanobolus vulcani* (Wilhelm *et al.*, 1986) have been described. Two infective phage particles were isolated from *Methanobrevibacter smithii*, the lytic phages PG (Baresi & Bertani, 1984) and

PMS1 (Knox & Harris, 1986) with genome sizes of 50 and 35 kb, respectively. The best characterized phage of the methanogenic branch is ΨM1 from the thermophilic *Methanobacterium thermoautotrophicum* Marburg (Meile *et al.*, 1989). This lytic phage contains a linear, circularly permuted 27.1 kb genome with a terminal redundancy of approximately 3 kb (Jordan *et al.*, 1989). Interestingly, besides the concatemeric precursor of the phage DNA which is packaged by a headful mechanism, multimers of the cryptic 4.4 kb plasmid pME2001 harboured by strain Marburg (Meile *et al.*, 1983) could also be isolated from ΨM1 particles (Meile *et al.*, 1989). This relaxed packaging machinery is probably responsible for the recently demonstrated general transduction mediated by ΨM1 (Meile *et al.*, 1990).

In the present study we report the isolation and initial characterization of two novel lytic archaeal phages, ΦF1 and ΦF3, capable of infecting different thermophilic strains of the genus *Methanobacterium*.

Methods

Bacterial strains and phages. The strains of *M. thermoformicum* and *M. thermoautotrophicum* used in this study are listed in Table 1.

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Table 1. *Methanogenic strains used in this study and their sensitivity to phage Φ F1 and Φ F3*

Strain*	Plasmid (kb)	Sensitivity to		Strain reference
		ΦF1†	ΦF3†	
<i>M. thermoformicum</i>				
Z-245 (DSM 3720)	pFZ1 (11)	+	—	Zhilina & Ilarionov (1984)
FTF (DSM 3012)	pFZ2 (11)	+	—	Touzel <i>et al.</i> (1988)
THF (DSM 3848)	pFV1 (13.5)	—	—	Zinder & Koch (1984)
FF1	—	+	—	Nölling <i>et al.</i> (1991)
FF3	—	+	+	Nölling <i>et al.</i> (1991)
CSM3	—	+	—	Nölling <i>et al.</i> (1993)
CB12 (DSM 3664)	—	—	—	Zhao <i>et al.</i> (1986)
SF-4	—	—	—	Yamamoto <i>et al.</i> (1989)
HN4	—	—	—	Nölling <i>et al.</i> (1993)
<i>M. thermoautotrophicum</i>				
ΔH (DSM 1053)	—	+	—	Zeikus & Wolfe (1972)
Marburg (DSM 2133)	pME2001 (4.4)	—	—	Fuchs <i>et al.</i> (1978)

* DSM, German Collection of Micro-organisms, Braunschweig, FRG.

† +, Efficiency of plating $> 10^{-1}$; —, efficiency of plating $< 10^{-5}$.

Cultivation of methanogenic strains in liquid cultures or on solidified 0.8% Gelrite plates (Roth) was performed as described previously (Nölling *et al.*, 1991) except that $0.2 \text{ g MgCl}_2 \text{ l}^{-1}$ was used. DNA of phage Ψ M1 was a gift from L. Meile (Department of Microbiology, ETH Zürich, Switzerland).

Isolation of methanogenic phages. For the isolation of phages Φ F1 and Φ F3, samples were taken anaerobically from an upflow anaerobic sludge-bed reactor operating at 55°C (Department of Environmental Engineering, University of Wageningen, The Netherlands) which had already served as source for the isolation of *M. thermoformicum* strains FF1 and FF3 (Nölling *et al.*, 1991). For enrichment of phages, 10 ml aliquots of the samples were sterilized by filtration on a $0.2 \mu\text{m}$ filter (Schleicher & Schuell) and added to 100 ml serum bottles containing 20 ml medium described by Nölling *et al.* (1991). These serum bottles were inoculated with 1 ml of exponentially grown cultures of either strain FF1 or FF3 and incubated for at least 2 d under H_2/CO_2 (4:1, v/v) at 55°C . Samples of the enrichment culture were then tested for plaque formation on a lawn of the indicator strains FF1 and FF3.

Propagation and titration of phages. For small-scale preparation of phage lysates, a single plaque was added to 100 ml serum bottles containing 20 ml medium (Nölling *et al.*, 1991) inoculated with 1 ml of exponentially grown cultures of the appropriate methanogenic host (about 5×10^7 cells ml^{-1}) and incubated for 1 or 2 d at 55°C under a H_2/CO_2 atmosphere in a shaking incubator at 150 r.p.m. Large quantities of phage lysate were obtained under similar conditions using 1 litre serum bottles containing 0.2 l medium inoculated with 0.2 ml phage lysate (approximately $10 \text{ p.f.u. ml}^{-1}$) and 1 ml of a methanogenic culture. Alternatively, high titre lysates ($10^9 \text{ p.f.u. ml}^{-1}$) were prepared from plate lysates obtained by flooding agar plates showing confluent lysis with 1.5 ml SM buffer (Sambrook *et al.*, 1989). For routine propagation of phages, phage lysates were prepared in 100 ml serum bottles as described above, sterilized by filtration through a $0.2 \mu\text{m}$ filter and stored anaerobically at 4°C .

Phage titres were determined by the soft agar overlay method essentially as described by Meile *et al.* (1989). Usually, 0.1 ml of (diluted) phage lysate was mixed with 0.3 ml exponentially growing cells and incubated for 20 min at room temperature to allow phage

adsorption. Soft agar (2.5 ml 0.7% agar) was added and the mixture was poured onto freshly prepared and dried Gelrite plates (Nölling *et al.*, 1991). The plates were incubated upside down at 55°C under a H_2/CO_2 atmosphere for 1–3 d.

Isolation of DNA. Phage lysates were centrifuged at 10000 g for 10 min and the supernatant was collected. DNAase and RNAase A (Boehringer Mannheim) were added to a final concentration of $0.2 \mu\text{g ml}^{-1}$, and the supernatant was gently stirred for 1 h at room temperature. Phage particles were precipitated by adjusting the supernatant to 1 M-NaCl and 10% (w/v) PEG 6000, followed by an overnight incubation at 4°C . The phage precipitate was recovered by centrifugation at 10000 g for 10 min at 4°C and gently resuspended in SM buffer. After addition of SDS and EDTA to a final concentration of 0.5% and 20 mM, respectively, the suspension was treated with proteinase K (0.1 mg ml^{-1} ; Boehringer Mannheim) for 1 h at 56°C . The DNA was deproteinized by several phenol/chloroform extractions followed by an extraction with chloroform. Finally, the DNA was precipitated with ethanol and dissolved in TE buffer (10 mM-Tris/HCl pH 8.0, 1 mM-EDTA).

Total DNA from strains of the genus *Methanobacterium* was isolated as described by Nölling *et al.* (1991).

Manipulation and hybridization of DNA. All restriction enzymes were purchased from Life Technologies and used according to the instructions of the supplier. *R. Mth*ZI and *R. Mth*TI endonuclease assays were performed as described earlier using extracts prepared from *M. thermoformicum* Z-245 (Nölling & de Vos, 1992b) and from *Escherichia coli* harbouring pURM4 Δ M with the cloned *mthTIR* gene from plasmid pFV1 of *M. thermoformicum* THF (Nölling & de Vos, 1992a), respectively. To remove histone-like proteins present in the extracts prepared from strain Z-245, *R. Mth*ZI samples were deproteinized prior to gel electrophoresis as described previously (Nölling & de Vos, 1992b).

For DNA–DNA hybridization, DNA was transferred from agarose gels to Hyperbond membranes by a capillary-blot procedure as specified by the supplier (Amersham). For preparation of DNA probes, either total phage DNA or DNA fragments isolated from agarose gels using the GeneClean kit (Bio 101) were labelled by nick-translation (Sambrook *et al.*, 1989) with [α - ^{32}P]dATP (Amersham).

Prehybridization and hybridization were performed as described earlier (Nölling *et al.*, 1991). Washing procedures were carried out in $2 \times \text{SSC}/0.1\%$ SDS at 55°C (medium stringent) and in $0.1 \times \text{SSC}/0.1\%$ SDS at 65°C (stringent) ($1 \times \text{SSC}$ is 150 mM-sodium chloride, 15 mM-sodium citrate).

Electron microscopy. Preparations of ΦF1 and ΦF3 were placed on Formvar-coated copper grids and stained with 1% (w/v) uranyl acetate. The electron microscope used for examination was a Philips CM12.

Results

Morphology of phage ΦF1 and ΦF3

We have previously reported the isolation of the *M. thermoformicum* strains FF1 and FF3 from an anaerobic, thermophilic digester (Nölling *et al.*, 1991). Both strains served as indicators for the enrichment and subsequent isolation of two phages from the same anaerobic digester, which accordingly were designated ΦF and ΦF3 .

Electron microscopy of negatively stained phage preparations revealed a similar morphology of phages ΦF1 and ΦF3 . Phage ΦF1 consists of an isometric, icosahedric capsid, approximately 70 nm in diameter and a non-flexible tail of approximately 160 nm in length and 20 nm wide (Fig. 1*a*). Phage ΦF3 is composed of an isometric, icosahedric head of approximately 55 nm in diameter and a tail 230 nm long and 9 nm wide which is flexible in contrast to that of phage ΦF1 (Fig. 1*b*). The ΦF3 tail consists of about 60 transverse segments (Fig. 1*b*). Since no shortened tails were detected, the tails of ΦF1 and ΦF3 are probably not contractile. The morphology of both ΦF1 and ΦF3 corresponds to that of Bradley's classification group B (Bradley, 1967).

Host specificity of phages ΦF1 and ΦF3

All known strains of *M. thermoformicum* and the *M. thermoautotrophicum* strains Marburg and ΔH were tested for sensitivity to phages ΦF1 and ΦF3 . As summarized in Table 1 the phages differ with respect to their host range. While phage ΦF3 could be propagated only on *M. thermoformicum* FF3, phage ΦF1 was found to infect a variety of strains including *M. thermoformicum* strains Z-245, FTF, FF1, FF3 and CSM3, and *M. thermoautotrophicum* ΔH . No plaque formation was observed when each of the other strains was used as an indicator, even after increasing the number of phages to approximately 10^5 p.f.u. per plate.

Lysates produced by ΦF1 on either host and ΦF3 lysates contained similar amounts of infective particles and titres of $1\text{--}5 \times 10^6$ p.f.u. ml^{-1} were obtained. Phage ΦF1 formed clear, small (< 1 mm in diameter) plaques that became visible after 2–3 days of incubation. The

clear plaques produced by phage ΦF3 were approximately 2 mm in diameter and could be detected after about 20 h of incubation. Storing the lysates produced by either phage in the presence of oxygen did not affect the phage titres.

Genomes of phages ΦF1 and ΦF3

The genomes of the phages ΦF1 and ΦF3 were double-stranded DNA molecules which served as good substrates for cleavage by 17 type II restriction endonucleases with different specificity. For the calculation of the genome sizes and for the construction of physical maps, we have used several commercially available restriction endonucleases and those produced by *M. thermoformicum* THF and Z-245, the GGCC-specific R.*Mth*TI (Nölling & de Vos, 1992*a*) and the CTAG-recognizing R.*Mth*ZI (Nölling & de Vos, 1992*b*), respectively.

The size of the ΦF1 genome was estimated to be 85 ± 5 kb by summing the length of the restriction fragments derived from digestion with *Sal*I (15 fragments; Fig. 2) and from a triple digest with *Xba*I–*Not*I–*Mlu*I (eight fragments). Further restriction analysis revealed no sites for *Sfi*I, one site for *Xba*I and *Mth*ZI (Fig. 2) and three sites for *Mlu*I (Fig. 2) and *Not*I. The latter three enzymes were used in double and triple digestions for the construction of a physical map which showed that ΦF1 contains a linear genome (Fig. 3). Incubation of ΦF1 DNA with *Mth*TI generated multiple fragments, the majority of which were smaller than 1 kb (data not shown). To examine whether ΦF1 contains cohesive ends, *Mlu*I-digested phage DNA was heated to 78°C for 10 min and immediately cooled on ice. Separation by agarose gel electrophoresis together with non-heated *Mlu*I-digested ΦF1 DNA did not reveal any difference in restriction pattern (data not shown).

Restriction digestion of phage ΦF3 DNA with *Sal*I and *Mth*ZI produced four and three fragments, respectively (Fig. 2). If the ΦF3 genome were linear, this would correspond to three *Sal*I and two *Mth*ZI sites for the ΦF3 genome which would generate six restriction fragments upon a double digestion with both endonucleases. However, we observed seven fragments derived from digestion of ΦF3 DNA with *Sal*I followed by incubation with *Mth*ZI (Fig. 2). Interpretation of these results led to the conclusion that phage ΦF3 contains a circular or terminally redundant linear genome. Mapping of the restriction sites for *Sst*I, *Hind*III, *Sal*I and *Mth*ZI confirmed this configuration of the ΦF3 genome which had a calculated size of 36 ± 2 kb (Fig. 3). Possible circularization of the ΦF3 genome does not seem to be mediated by cohesive ends since heated (78°C for 10 min) and non-heated *Sal*I digests of ΦF3 DNA

Fig. 1. Negatively stained Φ F1 (a) and Φ F3 (b) particles. Bars, 70 nm.

Fig. 2. Restriction analysis of DNA prepared from phage Φ F1 (a) and Φ F3 (b). (a) Approximately 0.3 μ g Φ F1 DNA undigested (lane 1), and digested with *Mth*ZI (lane 2), *Mlu*I (lane 3) and *Sal*I (lane 4). (b) Approximately 0.5 μ g Φ F3 DNA undigested (lane 1), and digested with *Sal*I (lane 2), *Sal*I-*Sst*I (lane 3), *Sal*I-*Sst*I-*Mth*ZI (lane 4), *Sal*I-*Mth*ZI (lane 5), *Sst*I-*Mth*ZI (lane 6), and *Mth*ZI (lane 7). Lanes s show *Hind*III-digested λ DNA as size marker.

Fig. 3. Physical maps of the linear genome of Φ F1 and the circular genome of Φ F3. Sizes are given in kb.

showed no differences in restriction pattern after separation by agarose gel electrophoresis (data not shown).

Hybridization of Φ F1 and Φ F3 DNA with DNA from other sources

Hybridization studies using radioactively labelled Φ F1 and Φ F3 DNA revealed no similarity to digested total

DNA from the *Methanobacterium* strains used in this study (data not shown). In addition, no cross-hybridization was observed between DNA from Φ F1, Φ F3 and Ψ M1 DNA (data not shown).

Since phage Ψ M1 was found to package multimers of plasmid pME2001 harboured by its host *M. thermoautotrophicum* Marburg (Meile *et al.*, 1989), we tested whether phage Φ F1 contained (fragments of) plasmid pFZ1 DNA from *M. thermoformicum* Z-245. For this purpose, we extracted DNA from phages propagated on strain Z-245 and the plasmid-free *M. thermoautotrophicum* Δ H and hybridized it, after digestion and subsequent blotting, to the three cloned *Kpm*I fragments of plasmid pFZ1 labelled by nick-translation (Nölling *et al.*, 1991). No hybridization was observed (data not shown) indicating that Φ F1 does not have the capacity to package pFZ1 DNA.

Discussion

Two novel lytic phages Φ F1 and Φ F3 capable of infecting different thermophilic strains of the archaeal genus *Methanobacterium* were described. Both phages Φ F1 and Φ F3 were composed of a polyhedral head and a tail similar to the morphology of the phages found for halophilic and methanogenic Archaea, with the exception of the virus-like particle of *Methanococcus voltae* A3 (Wood *et al.*, 1989). No hybridization was found between DNA of the new phages and DNA of Ψ M1, a previously identified methanogen phage (Meile *et al.*, 1991).

Phage Φ F1 contains a linear genome consisting of approximately 85 kb which is the largest genome so far reported for a methanogen phage. In contrast, DNA extracted from Φ F3 phage particles was approximately 36 kb in size and showed a circular or terminally redundant linear genome. A circular genome topology has also been found for the virus-like particle from *Methanococcus voltae* A3 which contains a 23 kb DNA molecule (Wood *et al.*, 1989) and the 15.5 kb genome of the virus SSV1 from *Sulfolobus shibatae* (Nadal *et al.*, 1986).

The phages Φ F1 and Φ F3 differ remarkably in their host range. In common to phage Ψ M1 of *M. thermoautotrophicum* Marburg, phage Φ F3 has a narrow host-range and could only be propagated on *M. thermoformicum* FF3 which served as indicator strain for the isolation of Φ F3. In contrast, phage Φ F1 was capable of infecting five strains of *M. thermoformicum* and *M. thermoautotrophicum* strain Δ H. This broad host-range of Φ F1 is relevant in the context of the recently reported phylogenetic studies on thermophilic members of the genus *Methanobacterium*. Based on DNA-DNA similarity (Touzel *et al.*, 1992) and 16S rRNA analysis (Nölling *et*

al., 1993), these studies provided evidence for the conclusions that (i) the species *M. thermoformicum* can be divided into two groups of strains, the Z-245 and CB12 groups, which may constitute different species, (ii) strain *M. thermoautotrophicum* Δ H belongs to the Z-245 group, and (iii) *M. thermoautotrophicum* Marburg is clearly different from either of those strains. The host range of Φ F1 that includes *M. thermoformicum* strains Z-245, FTF, CSM3, FF1 and FF3, and *M. thermoautotrophicum* strain Δ H, all of which are closely related members of the Z-245 group, supports the proposed different taxonomic position of these thermophilic *Methanobacterium* strains (Touzel *et al.*, 1992; Nölling *et al.*, 1993).

Phage Φ F1 failed, however, to infect *M. thermoformicum* strain THF which is a more distantly related member of the Z-245 group (Touzel *et al.*, 1992; Nölling *et al.*, 1993). Although strain THF could be a trivial phage-resistant mutant it is also possible that the penetrated Φ F1 DNA is inactivated by endonuclease attack of the plasmid-encoded, GGCC-recognizing type II restriction-modification (R/M) system *Mth*TI of strain THF (Nölling & de Vos, 1992a). If so, the R/M system of strain THF would be more efficient in protecting against phage Φ F1 infection than the R/M systems *Mth*ZI and *Mth*FI of strains Z-245 and FTF, respectively, that are specific for the sequence CTAG (Nölling & de Vos, 1992b). The extreme difference in the frequency of appropriate endonuclease target sites in the 80 kb genome of Φ F1, i.e. a single CTAG site versus multiple GGCC sites, would support this assumption (Figs 2 and 3). Although CTAG is one of the rarest occurring tetranucleotides in DNA of methanogens (J. Nölling, unpublished results) the exceptionally strong bias against CTAG found in the genome of Φ F1 could well be a result of counter-selection against restriction sites in the phage DNA in order to escape the host-controlled restriction barrier. Such an anti-restriction mechanism is one of the phage-derived defence strategies known to be utilized by certain phages of the bacterial domain to avoid restriction by the host (Krüger & Bickle, 1983).

The specificity of phage Φ F3 for *M. thermoformicum* strain FF3 as its only host was unexpected since strain FF3 belongs to the group of closely related strains that serve as host for Φ F1. The existence of a type II restriction barrier is unlikely since, in contrast to *M. thermoformicum* strains THF, Z-245 and FTF, extracts prepared from strains FF3, FF1 and CSM3 and *M. thermoautotrophicum* Δ H did not contain detectable site-specific endonuclease activities (Nölling & de Vos, 1992b). These findings suggest that phage Φ F3 depends on a specific factor which is present only in strain FF3 or that the other strains of the Z-245 group contain

additional phage defence mechanisms such as type I or III R/M systems.

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References

- BARESI, L. & BERTANI, G. (1984). Isolation of a bacteriophage for a methanogenic bacterium. In *Abstracts of the Annual Meeting of the American Society for Microbiology*, New Orleans, 174, p. 133. Washington, DC: American Society for Microbiology.
- BRADLEY, D. E. (1967). Ultrastructure of bacteriophages and bacteriocins. *Bacteriological Reviews* **31**, 230–314.
- FUCHS, G., STUPPERICH, E. & THAUER, R. K. (1978). Acetyl CoA, a central intermediate of autotrophic CO₂ fixation in *Methanobacterium thermoautotrophicum*. *Archives of Microbiology* **127**, 267–272.
- JORDAN, M., MEILE, L. & LEISINGER, T. (1989). Organization of *Methanobacterium thermoautotrophicum* bacteriophage Ψ M1 DNA. *Molecular and General Genetics* **220**, 161–164.
- KNOX, M. R. & HARRIS, J. E. (1986). Isolation and characterization of a bacteriophage from *Methanobrevibacter smithii*. In *Abstracts of the XIV International Congress of Microbiology*, P.G3-8, p. 240. Manchester, UK: International Union of Microbiology.
- KRÜGER, D. H. & BICKLE, T. A. (1983). Bacteriophage survival: multiple mechanisms for avoiding the deoxyribonucleic acid restriction systems of their hosts. *Microbiological Reviews* **47**, 345–360.
- MEILE, L., KIENER, A. & LEISINGER, T. (1983). A plasmid in the archaeobacterium *Methanobacterium thermoautotrophicum*. *Molecular and General Genetics* **191**, 480–484.
- MEILE, L., ABENDSCHEIN, P. & LEISINGER, T. (1990). Transduction in the archaeobacterium *Methanobacterium thermoautotrophicum* Marburg. *Journal of Bacteriology* **172**, 3507–3508.
- MEILE, L., JENAL, U., STUDER, D., JORDAN, M. & LEISINGER, T. (1989). Characterization of Ψ M1, a virulent phage of *Methanobacterium thermoautotrophicum* Marburg. *Archives of Microbiology* **152**, 105–110.
- NADAL, M., MIRAMBEAU, G., FORTERRE, P., REITER, W.-D. & DUGUET, M. (1986). Positively supercoiled DNA in a virus-like particle of an archaeobacterium. *Nature, London* **321**, 256–258.
- NÖLLING, J. & DE VOS, W. M. (1992a). Characterization of the archaeal, plasmid-encoded type II restriction-modification system *Mth*II from *Methanobacterium thermoformicum* THF: homology to the bacterial *Ngo*PII system from *Neisseria gonorrhoeae*. *Journal of Bacteriology* **174**, 5719–5726.
- NÖLLING, J. & DE VOS, W. M. (1992b). Identification of the CTAG-recognizing restriction-modification systems *Mth*ZI and *Mth*FI from *Methanobacterium thermoformicum* and characterization of the plasmid-encoded *mth*ZIM gene. *Nucleic Acids Research* **20**, 5047–5052.
- NÖLLING, J., FRIJLINK, M. & DE VOS, W. M. (1991). Isolation and characterization of plasmids from different strains of *Methanobacterium thermoformicum*. *Journal of General Microbiology* **137**, 1981–1986.
- NÖLLING, J., HAHN, D., LUDWIG, W. & DE VOS, W. M. (1993). Phylogenetic analysis of thermophilic *Methanobacterium* sp.: evidence for a common formate-utilizing ancestor. *Systematic and Applied Microbiology* (in the Press).
- SAMBROOK, J., FRITSCH, E. F. & MANIATIS, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- TOUZEL, J. P., PETROFF, D., MAESTROJUAN, G. M., PRENSIER, G. & ALBAGNAC, C. (1988). Isolation and characterization of a thermophilic *Methanobacterium* able to use formate, the strain FTF. *Archives of Microbiology* **149**, 291–296.
- TOUZEL, J. P., CONWAY DE MACARIO, E., NÖLLING, J., DE VOS, W. M., ZHILINA, T. & LYSSENKO, A. M. (1992). DNA relatedness among some thermophilic members of the genus *Methanobacterium*: emendation of the species *Methanobacterium thermoautotrophicum* and rejection of *Methanobacterium thermoformicum* as a synonym of *Methanobacterium thermoautotrophicum*. *International Journal of Systematic Bacteriology* **42**, 408–411.
- WAIS, A. C., KON, M., MACDONALD, R. E. & STOLLAR, B. D. (1975). Salt-dependent bacteriophage infecting *Halobacterium cutirubrum* and *H. halobium*. *Nature, London* **256**, 314–315.
- WILHARM, T., THOMM, M. & STETTER, K. O. (1986). Genetic analysis of plasmid pMP1 from *Methanobacterium vulcani*. *Systematic and Applied Microbiology* **7**, 401.
- WOESE, C. R., KANDLER, O. & WHEELIS, M. J. (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria and Eucarya. *Proceedings of the National Academy of Sciences of the United States of America* **87**, 4576–4579.
- WOOD, A. G., WHITMAN, W. B. & KONISKY, J. (1989). Isolation and characterization of an archaeobacterial viruslike particle from *Methanococcus voltae* A3. *Journal of Bacteriology* **171**, 93–98.
- YAMAMOTO, K., TACHIBANA, A., DHAVISES, G., TANAKA, T., TANIGUCHI, M. & OI, S. (1989). Characterization of a thermophilic formate-utilizing methanogen. *Methanobacterium thermoformicum* strain SF-4. *Agricultural and Biological Chemistry* **53**, 533–534.
- ZHAO, Y., ZHANG, H., BOONE, D. & MAH, R. A. (1986). Isolation and characterization of a fast-growing, thermophilic *Methanobacterium* species. *Applied and Environmental Microbiology* **52**, 1227–1229.
- ZEIKUS, J. G. & WOLFE, R. S. (1972). *Methanobacterium thermoautotrophicum* sp. n., an anaerobic, autotrophic, extreme thermophile. *Journal of Bacteriology* **109**, 707–713.
- ZHILINA, T. N. & ILARIONOV, S. A. (1984). Characteristics of formate assimilating methane bacteria and description of *Methanobacterium thermoformicum* sp. nov. *Microbiology* **53**, 647–651 (English translation of *Mikrobiologiya* **53**, 785–790).
- ZILLIG, W., REITER, W.-D., PALM, P., GROPP, F., NEUMANN, H. & RETTENBERGER, M. (1988). Viruses of archaeobacteria. In *The Bacteriophages*, vol. 1, pp. 517–558. Edited by R. Calendar. New York: Plenum Press.
- ZINDER, S. & KOCH, M. (1984). Non-acetoclastic methanogenesis from acetate: acetate oxidation by a thermophilic syntrophic coculture. *Archives of Microbiology* **138**, 263–272.