The DNA of certain species of halophilic and methanogenic archaeabacteria is dam methylated, as shown by restriction endonuclease sensitivities. The Dam+ phenotype appears to be confined to particular taxonomic groupings defined by DNA : rRNA hybridization or 16S RNA oligonucleotide cataloguing.

**INTRODUCTION**

DNA adenine methylase (dam methylase) transfers methyl groups from S-methyladenosylmethionine to the N6 position of the adenine residue within the DNA sequence GATC (Geier & Modrich, 1979; Hattman et al., 1978). This enzyme plays no part in restriction modification, but may be involved in post-replication mismatch repair, since mismatched bases are preferentially removed from the unmethylated strand of heteroduplex DNA (Wagner & Meselson, 1976; Glickmann et al., 1978; Lu et al., 1983; Pukkila et al., 1983). A role in DNA replication has also been proposed, in view of the high frequency of occurrence of GATC sequences at the origin of replication in species producing the enzyme (Meijer et al., 1979; Zyskind & Smith, 1980; Cleary et al., 1982). Zyskind & Smith (1980) suggested that this may serve to direct the mismatch repair enzymes to the newly replicated origin, thus ensuring greater conservation of the sequence. GATC sequences also frequently occur at the ends of Okazaki fragments, which may also suggest a role in DNA replication (Marinus, 1976; Gomez-Eichelmann & Lark, 1977).

The presence of methylated GATC sequences, and hence the dam methylase, may be shown by the use of the isoschizomer restriction endonucleases MboI, Sau3AI, and DpnI (Sussenbach et al., 1976; Gelinaset al., 1976; Lacks & Greenberg, 1977). All recognize the sequence GATC, but are affected in different ways by methylation of the A residue. MboI is inhibited by methylation (Dreiseikelman et al., 1979), Sau3AI is unaffected, and DpnI will only cut methylated sequences (McClelland, 1983). Strains whose DNA is cut by Sau3AI and MboI, but not DpnI, have no methylation of the recognition sequence and can be considered Dam−, whereas strains whose DNA is cut by Sau3AI and DpnI, but not MboI, have methylation of the A residue and are therefore Dam+.

Barbeyron et al. (1984) reported the Dam+ phenotype in members of nine genera of the families Enterobacteriaceae, Vibrionaceae and Pasteurellaceae. This phenotype was also found in five cyanobacteria, and partial endonuclease reactions were observed with two archaeabacteria: the extreme halophile Halobacterium saccharovorum, and a Methanobacterium strain. Barbeyron et al. (1984) argued that dam methylation has appeared independently in bacterial evolution at least twice and that in the enterobacterial lineage it has appeared relatively late. Hattman et al. (1985) however, suggested a common evolutionary origin for all dam methylases in view of the amino acid homology between the enzymes produced by Escherichia coli, Streptococcus pneumoniae and bacteriophage T4.
The unequivocal demonstration of \textit{dam} methylation in several groups of archaeabacteria would indicate an early evolutionary origin for this DNA strand identification system. In this study we present evidence that the DNAs of several species of archaeabacteria are \textit{dam} methylated, and that the patterns observed correlate with taxonomic groupings.

**METHODS**

\textbf{Strains and culture conditions.} Strains and their sources are listed in Table 1. Members of the genera \textit{Halobacterium} and \textit{Halococcus} were grown at 37 °C in the light (Gallenkamp illuminated incubator) in liquid shaken culture in the medium of Payne et al. (1960), with the exceptions of \textit{Hb. saccharovorum}, which was grown at 45 °C, \textit{Hb. volcanii}, which was grown on the medium of Mullakhanbai & Larsen (1975), and \textit{Hb. sodomense}, which was grown in the medium of Oren (1983). An extremely halophilic new isolate 2SR was grown at 40 °C on a medium containing: NaCl 250 g l\(^{-1}\), MgSO\(_4\), 7H\(_2\)O 20 g l\(^{-1}\), trisodium citrate 3 g l\(^{-1}\), KCl 2 g l\(^{-1}\), yeast extract (Difco) 10 g l\(^{-1}\), and vitamin assay Casamino acids (Difco) 7.5 g l\(^{-1}\), pH 7.5.

\textit{Natronobacterium} and \textit{Natronococcus} spp. were grown at 37 °C in the medium of Tindall et al. (1980). \textit{Methanococcus} and \textit{Methanogenium} spp. were grown to stationary phase at 37 °C and 25 °C respectively, in medium 3 of Balch et al. (1979) supplemented with 0.001 g resazurin l\(^{-1}\). The trace mineral solution additionally contained 0.01 g NiCl\(_2\) l\(^{-1}\) and 0.01 g NaSeO\(_4\) l\(^{-1}\). All other methanogens were grown to stationary phase at 37 °C, in M3 medium of the following composition (g l\(^{-1}\)):

- Yeast extract, 0.9
- (NH\(_4\))\(_2\)SO\(_4\), 0.9
- K\(_2\)HPO\(_4\), 0.9
- KH\(_2\)PO\(_4\), 0.45
- CaCl\(_2\), 0.35
- MgSO\(_4\), 0.2
- FeSO\(_4\), 0.3
- Na\(_2\)EDTA, 0.01
- plus trace element solution (as above), 10 ml l\(^{-1}\). All media were boiled out and cooled under 80% N\(_2)/20% CO\(_2\), and Na\(_2\)CO\(_3\) (2 g l\(^{-1}\)) was added to the M3 medium. The pH of each medium was adjusted to 6.8 before reduction by addition of cystine hydrochloride (0.4 g l\(^{-1}\)) and Na\(_2\)S. 9H\(_2\)O (0.4 g l\(^{-1}\)).

After inoculation, the dead-space gas was replaced with 80% H\(_2)/20% CO\(_2\) at 2 atm pressure and replenished daily, except in the case of \textit{Methanospirillum} spp., where the growth substrate was CH\(_3\)OH or 124 mM-monomethylamine (\textit{Ms. mazei}) added prior to inoculation at 1% (w/v). Cultures were grown in 400 ml amounts in 1-litre Duran bottles modified to incorporate a glass tube fitted with a butyl-rubber septum similar to that described by Balch et al. (1979); incubation was initially stationary for 24 h followed by shaking at 150 r.p.m.

\textbf{Cell lysis and preparation of DNA.} \textit{Methanococcus} and \textit{Methanogenium} and \textit{Methanobacterium} cells were harvested in the late exponential phase, resuspended in 10 mM-Tris pH 8 containing 100 mM-EDTA and lysed by the addition of SDS to a final concentration of 1% (w/v).

\textit{Methanobacterium, Methanobrevibacter} and \textit{Methanomicrobium} cells were harvested as above, but broken by passage through a French pressure cell. After breakage was judged (microscopically) to have occurred, SDS was added to 1% (w/v).

\textit{Ms. barkeri} cells release protoplasts in the late exponential phase (Davis & Harris, 1985). These were harvested and lysed as above.

\textit{Ms. mazei} cells spontaneously disaggregate and release osmotically sensitive cells during growth on monomethylamine. These cells were harvested and lysed as above.

The methods of Chater et al. (1982) were used to purify DNA from all lysates of methanogens. Halophile and haloalkaliphile DNA was prepared as described by Tindall et al. (1984). \textit{Sulfolobus solfataricus} DNA was a kind gift from Dr M. De Rosa, Instituto per la Chimica di Molecole di Interesse Biologico del CNR, Naples, Italy.

\textbf{Restriction endonuclease digests.} Restriction endonucleases were obtained from Bethesda Research Laboratories (MboI and Sau3AI) and Boehringer Mannheim (DpnI). Conditions used were those recommended by the manufacturers.

\textbf{Agarose gel electrophoresis.} DNA samples were fractionated on 0-75% agarose (HGT-Miles) run in ELFO buffer (40 mM-Tris/acetic acid pH 7.7, 1-24 mM-EDTA).

\section*{RESULTS AND DISCUSSION}

Fig. 1 shows examples of \textit{Dam}+ and \textit{Dam}− reactions and Table 1 indicates that the \textit{Dam}+ phenotype is exhibited by examples of both halophilic and methanogenic archaeabacteria. In the extreme halophiles, \textit{dam} methylation is confined to the members of one DNA/16S rRNA homology group as defined by Ross & Grant (1985). The newly isolated strain 2SR, which chemotaxonomic studies place in the same homology group (D. Lodwick, unpublished), is also \textit{dam} methylated (Table 1). Although we have not surveyed all the known species of methanogenic archaeabacteria, the preliminary results presented in Table 1 suggest that the \textit{Dam}+ phenotype is confined to members of the genera \textit{Methanogenium}, \textit{Methanospirillum}, \textit{Methanobacterium} and \textit{Methanolobus}.

\textit{Dam}+ phenotypes can be found in members of the families \textit{Enterobacteriaceae} and
Vibrionaceae, bacteriophages T1, T2 and T4, Moraxella bovis, Streptococcus pneumoniae, and certain cyanobacteria (Hattman et al., 1985; Brooks et al., 1983; Barbeyron et al., 1984; Mannarelli et al., 1985). The work of Hattman et al. (1985) has demonstrated amino acid homology between the genes from E. coli, bacteriophage T4 and S. pneumoniae, and despite the failure of Brooks et al. (1983) to hybridize the E. coli dam gene to DNA from Anabaena variabilis, it seems likely that the genes share a common evolutionary origin. Barbeyron et al. (1984) have produced a phylogeny of Dam+ and Dam− organisms and speculated that the Dam+ phenotype appeared in the enterobacterial lineage quite recently. They were unable to demonstrate unambiguous evidence for dam methylation in the archaebacteria, although partial sensitivity to DpnI was observed for DNA from two archaebacterial examples. It is clear from the results presented here that dam methylation is relatively widespread in archaebacteria, and that the distribution of the Dam+ phenotype is consistent with the presently perceived phylogeny of the group. We have recently speculated that the DNA/16S rRNA homology group of halophiles reported here to be Dam+ (Table 1) constitutes a new genus (Grant & Ross, 1986).

The presence of dam methylation in two of the main archaebacterial lineages suggests an ancient origin for the process, and tends to support the suggestion of Hattman et al. (1985) that the origin of the dam genes predates the divergence of Gram-positive and Gram-negative bacteria. The halophiles are as phylogenetically distant from the methanogens as enterobacteria are from cyanobacteria (Stackebrandt & Woese, 1981), and it thus seems less than likely that the Dam+ phenotype would have arisen independently in several distinct groups of prokaryotes. Further homology studies between different dam genes, including those from archaebacteria, are required to resolve the question.
Table 1. *Dam phenotype of 36 strains of archaebacteria*

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain*</th>
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<td><em>Sulfobolus sulfatarius</em></td>
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</table>

* ATCC, American Type Culture Collection (Maryland, USA); CCM, Czechoslovak Collection of Microorganisms (Brno, Czechoslovakia); DSM, Deutsche Sammlung von Mikroorganismen (Munich, FRG); NCMB, National Collection of Marine Bacteria (Aberdeen, UK).

† +, Digestion; −, no digestion.
‡ This strain resembles the lost strain of *Halobacterium marismortui* (Elazari-Volcani, 1957) and was kindly donated by Dr M. Ginzburg, Jerusalem, Israel.
§ New extremely halophilic isolate.

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