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Genomic Stability in the Archaeae *Haloferax volcanii* and *Haloferax mediterranei*

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Through hybridization of available probes, we have added nine genes to the macrorestriction map of the *Haloferax mediterranei* chromosome and five genes to the contig map of *Haloferax volcanii*. Additionally, we hybridized 17 of the mapped cosmid clones from *H. volcanii* to the *H. mediterranei* genome. The resulting 35-point chromosomal comparison revealed only two inversions and a few translocations. Forces known to promote rearrangement, common in the haloarchaea, have been ineffective in changing global gene order throughout the nearly 10^7 years of these species' divergent evolution.

One of the most notable characteristics of extremely halophilic archaea is their genetic instability (4, 11, 12, 27). The haloarchaea are rich in insertion sequences which can disrupt genes at frequencies as high as 10^{-2} in the case of *Halobacterium salinarium* (32, 43). Most of the activity of these insertion sequences is confined to plasmid DNA or to FII DNA (which has a lower moles percent G+C content) (13, 28, 31), but chromosomal genes are not spared from disruption. The *bop* gene, for instance, is inactivated by at least eight different types of insertion sequences (11, 27), resulting in a combined risk of about 10^{-4} per generation (32). *H. salinarium* is known to possess hundreds of insertion sequences, in dozens of families (35). The resulting transpositional cost to the cell is compounded by the potential recombinational chaos mediated by interaction between members of each insertion sequence family. It has been suggested that a genomically pure clone of *H. salinarium* cannot be grown because of continual rearrangements which occur in plasmid DNA (27, 30, 36).

The genus *Haloferax* is not as severely infested with insertion sequences as is *H. salinarium*. Nevertheless, *Haloferax volcanii* possesses at least 49 copies of the ISH51 family distributed throughout the genome (8), and there is good evidence for the existence of several other types of repeated sequences as well (35, 37). Though *H. volcanii* is not as prone to genetic disruption as is *H. salinarium*, neither is it immune. Less is known about the number or distribution of insertion sequences in *Haloferax mediterranei*. Interestingly, the populous ISH51/27 family shared by *H. volcanii* and *H. salinarium* (29) is absent from *H. mediterranei* (37). Regardless, repeated sequences which can potentially facilitate genomic rearrangement are present (1).

With physical and genetic maps available, we can now begin to address genomic stability in the haloarchaea. The first researchers to assess the degree of rearrangement in the haloarchaeal chromosome by using a comprehensive comparative mapping approach (15) found that much of the *H. salinarium* chromosome is conserved in structure among several strains, despite different complements of repetitive sequences in their genomes. Differences in the maps were observed to be confined to a few discrete, hypervariable blocks. *H. salinarium* NRC-1 and *H. salinarium* S9 maintain hundreds of insertion sequences within their genomes, and yet gene order is preserved relative to that of *H. salinarium* GRB, which contains virtually no repetitive sequences. The work was a clear demonstration of the existence of map inertia in the haloarchaea, at least over the short period of time—evidenced by a high level of conservation of restriction sites—since the strains diverged from one another.

Two other haloarchaeal genomic comparisons, spanning larger time scales, are currently feasible. Comparison of the *H. salinarium* GRB (40) and *H. volcanii* DS2 (6) contig maps will help us to examine genomic structural divergence at the genus level. Before undertaking this project, however, it was useful to assess genomic stability at the species level. This paper focuses on a comparison of the contig map of the *H. volcanii* DS2 genome (6) and the chromosomal macrorestriction map of *H. mediterranei* ATCC 33500 (1, 25), which we have here supplemented with additional loci to facilitate alignment. Numerical taxonomy (41) and DNA hybridization studies (14) clearly categorize *H. volcanii* and *H. mediterranei* as distinct species. A 98.4% similarity in 16S rRNA sequences (18) implies a divergence time of about 80 million years (26). We show here that despite sufficient opportunity for genomic rearrangement, the chromosomal maps of *H. volcanii* and *H. mediterranei* are chiefly congruent.

Comparison of the maps. Restriction maps are mutable, and they reflect sequence divergence. The *Bam*HI maps of the *H. volcanii* and *H. mediterranei* chromosomes are consequently dissimilar (Fig. 1), but this is not necessarily a result of genomic rearrangement. In order to examine map stability, we had to compare gene orders. Since we had only seven shared loci from our previous mapping efforts (1, 6), it was necessary to augment the number of these loci in order to improve the resolution. We first elected to complete the mapping by hybridization of available cloned genes as much as possible. With dot blots or Southern blots (40) of *H. volcanii* cosmid clones, we hybridized (24, 40) probes representing *dps*, *gyrA*, *ileS*, a *myc*-like gene, and the S10 ribosomal operon. With pulsed-field gel blots (24) of *H. mediterranei* genomic DNA, we hybridized (40) probes

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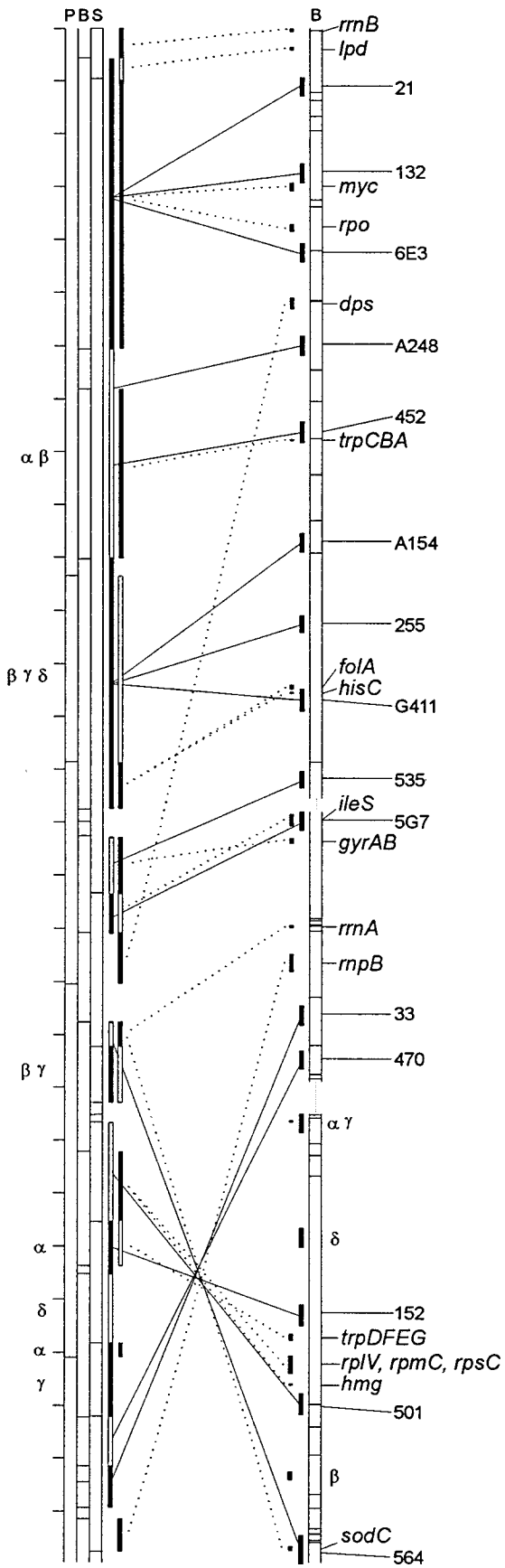

for *dps*, *hisC*, *hmg*, *ileS*, *lpd*, *rplAJL*, *rpo*, *trpCBA*, and *trpDFEG*. We thus added nine genetic loci to the *H. mediterranei* macrorestriction map and five to the contig map of *H. volcanii* (Table 1 and Fig. 1).

The ordered cosmid clone collection representing the *H. volcanii* genome (6) supplied ideal physical markers to supplement the limited number of genetic loci available. We chose 17 *H. volcanii* cosmid clones, spaced evenly around the chromosome, to hybridize (24) with *H. mediterranei* macrorestriction fragments. These cosmids were not known to carry repeated sequences (8), so that we might avoid extraneous cross hybridization. At high stringency, most of the cosmid probes produced a unique signal. The multiple signals produced by cosmids 686 and 464 (Fig. 2) arose either from transpositional fragmentation of the sequences within these cosmids or from events of duplicative transposition. Interestingly, the single-copy *csg* locus from *H. volcanii* maps to three places on the *H. mediterranei* chromosome (1). However, cosmid G86, which includes *csg*, hybridizes convincingly to only one of *csg*'s *H. mediterranei* residences. We interpret this to mean that the lower-specific-activity whole cosmid probe requires more extensive lengths of sequence to hybridize visibly. Therefore, we can conclude that there are three regions of the *H. mediterranei* chromosome that have extensive homology with G86 and that there are two other regions that have specific homology with *csg*. Duplicative transposition of *csg* is apparent; we cannot rule out other duplicative or nonduplicative transpositions involving short sequences such as those of single genes based on the cosmid-to-chromosome hybridizations, though we can be sure that the bulk of each cosmid's homology has been accurately mapped. Evidence for an event of nonduplicative transposition was observed in the case of one of our gene probes, that for *dps*.

An alignment of the enriched chromosomal maps, showing all shared loci derived from this work and from previous work, reveals an unexpected congruence (Fig. 1). When we found multicopy loci, one copy always matched the corresponding location on the other chromosome. Thus, apart from a large inversion presumably at the pair of inverted (6) *rrn* loci, another small inversion involving *ileS* and *gyrAB*, and a few transpositions, the chromosomes are colinear. Previously, during mapping of the *H. volcanii* genome (5), a hybrid cosmid clone which suggested recombination between the *rrn* loci was found (3). This large inversion may therefore not be a stable difference between the chromosomes.

Forces affecting rearrangement. Insertion sequences are known to cause frequent and extensive rearrangement of plasmid DNA in *H. salinarium*, and they can consequently alter plasmid-encoded phenotypes (27, 32, 43). Most transpositional activity is phenotypically invisible (36), however, which underscores the importance of insertion sequences in the evolution

FIG. 1. Comparison of the chromosomal maps of *H. volcanii* and *H. mediterranei*. Both chromosomes are circular and are of the same length, 2.9 Mbp. On the left, the macrorestriction map of the *H. mediterranei* chromosome is shown, with sites for *Pac*I (P), *Bam*HI (B), and *Swa*I (S). On the right, the *Bam*HI map of the *H. volcanii* chromosome is displayed. Dotted interruptions in this latter map indicate regions unmapped for *Bam*HI. Genes and *H. volcanii* cosmid clones with unique positions in the *H. mediterranei* map are linked to the *H. volcanii* map by dotted and solid lines, respectively. Genes and cosmid clones mapping to several locations on the *H. mediterranei* chromosome—*csg* (α), *rplAJL* (β), cosmid G86 (γ), and cosmid 464 (δ)—are shown as symbols without connections (see text). Genes were mapped to *Bam*HI, *Pac*I, and *Swa*I *H. mediterranei* fragments, whereas cosmids were mapped only to *Bam*HI and *Swa*I fragments. When loci could map anywhere within a restriction fragment, we traced lines to the center of the fragment. Tick marks on the scale bar on the left are placed at 100-kbp increments.

TABLE 1. Locations of genetic markers newly mapped in this study

Gene or operon	Description	*H. volcanii* cosmid(s)[a]	*H. mediterranei Bam*HI/*Swa*I/*Pac*I bands[b]	Reference
dps	DPS family heat shock	456 and A210	B9b/S3/P3	39
gyrA	DNA gyrase, subunit A	547		16
hisC	Histidinol phosphate aminotransferase		B2/S1/P3	9
hmg	3-hydroxy-3-methylglutaryl coenzyme A reductase		B7/S7/P2	21
ileS	Isoleucyl tRNA synthetase	5G7	B8/S3/P3	10
lpd	Dihydrolipoamide dehydrogenase	126[c]	B1/S11/P1	42
myc	Protein homologous to myc product	460		2
rp1AJL	Ribosomal protein A operon		B2/S1/P4, B3/S1/P1, and B6/S10/P2	17
rp1V, *rpmC*, *rpsC*	Ribosomal protein S10 operon	D57 and 266		38
rpo	RNA polymerase operon		B1/S1/P1	23
trpCBA	Tryptophan biosynthesis		B3/S1/P1	20
trpDFEG	Tryptophan biosynthesis		B7/S5/P2	22

[a] See reference 6.
[b] See reference 1.
[c] See reference 41a.

of haloarchaeal genome structure—there must be more underlying activity than we can observe through occasional phenotypic alterations. There is strong physical evidence for such disruptive potential in *Haloferax* spp. as well (8, 35, 37). We have discovered, though, that this destabilizing pressure has been largely ineffective in changing chromosomal organization in *Haloferax* spp. as far as the resolution of our comparison could show. A few minor differences are all that distinguish the current maps. The genetic core of the *Haloferax* genome, its chromosome, is remarkably stable. Plasmids, which can vary considerably (6, 24, 25, 34), remain the predominant seat of haloarchaeal genomic diversity.

Powerful stabilizing forces must exist in the haloarchaeal chromosome to maintain a particular gene order. We know little about the significance of gene order in a replicon. A catalog of genetic functions seems to be insufficient to describe the genome of an organism, since a highly conserved genetic map implies that the arrangement of genes is important as well. In *Escherichia coli* and *Salmonella typhimurium*, for which the most data exist (7, 19, 33), chromosome structure seems to be influenced by the distance and orientation of genes relative to the origin of DNA replication, by the desirability of terminating bidirectional replication 180° from the origin, by the need to maintain a certain polarity of sequences near the terminus of replication, and by a contextual sensitivity of gene expression. We know nothing about gene orientation, nucleoid structure, or the mechanics of chromosomal replication in the haloarchaea, but it is likely that similar forces influence gene location. In the haloarchaea, plasmid DNA seems to be less constrained by the factors which control the structure of the chromosome. Perhaps herein lie additional clues which will help us to discover what shapes a genome.

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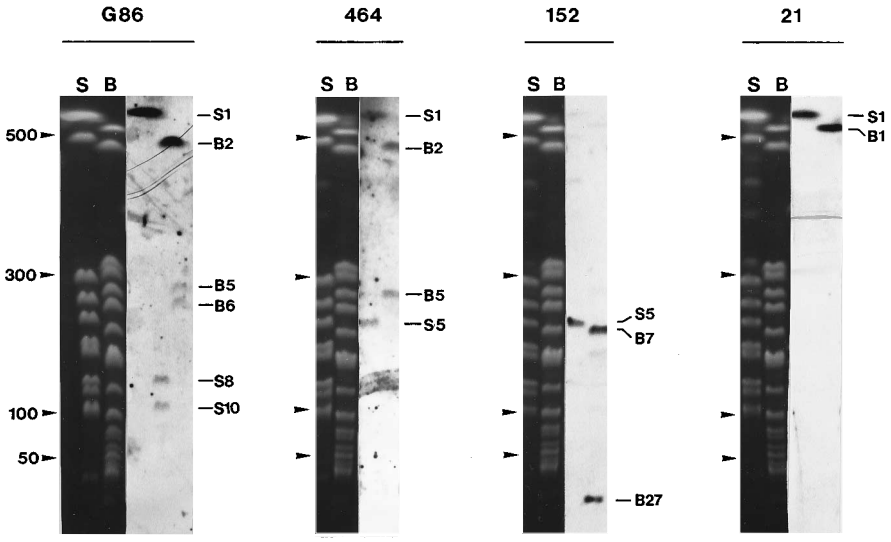

FIG. 2. Southern hybridization of *H. volcanii* cosmid clones with genomic DNA from *H. mediterranei*. Clones G86, 464, 152, and 21 were among the 17 cosmids used as probes in hybridizations with pulsed-field gel-fractionated *Swa*I (S) and *Bam*HI (B) genomic digests of *H. mediterranei*. G86 and 464 map to multiple chromosomal locations, whereas all other cosmids, including 152 and 21, map to single locations. The contour-clamped homogeneous electric field gels shown were run for 40 h at 10 V/cm, with a switching time of 10 s. As size standards, we used lambda concatemers and *Saccharomyces cerevisiae* YN295 chromosomes. Sizes shown on the left are in kilobase pairs.

REFERENCES

1. **Antón, J., P. López-García, J. P. Abad, C. L. Smith, and R. Amils.** 1994. Alignment of genes and *Swa*I restriction sites to the *Bam*HI genomic map of *Haloferax mediterranei*. FEMS Microbiol. Lett. **117:**53–60.
2. **Ben-Mahrez, K., W. Sougakoff, M. Nakayama, and M. Kohiyama.** 1988. Stimulation of an alpha like DNA polymerase by v-*myc* related protein of *Halobacterium halobium*. Arch. Microbiol. **149:**175–180.
3. **Charlebois, R. L.** Unpublished data.
4. **Charlebois, R. L., and W. F. Doolittle.** 1989. Transposable elements and genome structure in halobacteria, p. 297–307. *In* M. Howe and D. Berg (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
5. **Charlebois, R. L., J. D. Hofman, L. C. Schalkwyk, W. L. Lam, and W. F. Doolittle.** 1989. Genome mapping in halobacteria. Can. J. Microbiol. **35:**21–29.
6. **Charlebois, R. L., L. C. Schalkwyk, J. D. Hofman, and W. F. Doolittle.** 1991. A detailed physical map and set of overlapping clones covering the genome of the archaebacterium *Haloferax volcanii* DS2. J. Mol. Biol. **222:**509–524.
7. **Charlebois, R. L., and A. St. Jean.** J. Mol. Evol., in press.
8. **Cohen, A., W. L. Lam, R. L. Charlebois, W. F. Doolittle, and L. C. Schalkwyk.** 1992. Localizing genes on the map of the genome of *Haloferax volcanii*, one of the Archaea. Proc. Natl. Acad. Sci. USA **89:**1602–1606.
9. **Conover, R. K., and W. F. Doolittle.** 1990. Characterization of a gene involved in histidine biosynthesis in *Halobacterium* (*Haloferax*) *volcanii*: isolation and rapid mapping by transformation of an auxotroph with cosmid DNA. J. Bacteriol. **172:**3244–3249.
10. **Daniels, C. J.** 1992. Personal communication.
11. **DasSarma, S.** 1989. Mechanisms of genetic variability in *Halobacterium halobium*: the purple membrane and gas vesicle mutations. Can. J. Microbiol. **35:**65–72.
12. **Derkacheva, N. I., V. K. Kagramanova, and S. Mankin.** 1993. Genetic variability in halophilic archaebacteria (a review). Mol. Biol. **27:**287–295.
13. **Ebert, K., and W. Goebel.** 1985. Conserved and variable regions in the chromosomal and extrachromosomal DNA of halobacteria. Mol. Gen. Genet. **200:**96–102.
14. **Gutiérrez, M. C., A. Ventosa, and F. Ruiz-Berraquero.** 1989. DNA-DNA homology studies among strains of *Haloferax* and other halobacteria. Curr. Microbiol. **18:**253–256.
15. **Hackett, N. R., Y. Bobovnikova, and N. Heyrovska.** 1994. Conservation of chromosomal arrangement among three strains of the genetically unstable archaeon *Halobacterium salinarium*. J. Bacteriol. **176:**7711–7718.
16. **Holmes, M. L., and M. L. Dyall-Smith.** 1991. Mutations in DNA gyrase result in novobiocin resistance in halophilic archaebacteria. J. Bacteriol. **173:**642–648.
17. **Itoh, T.** 1988. Complete nucleotide sequence of the ribosomal 'A' protein operon from the archaebacterium, *Halobacterium halobium*. Eur. J. Biochem. **176:**297–303.
18. **Kamekura, M., and Y. Seno.** 1992. Nucleotide sequences of 16S rRNA encoding genes from halophilic archaea *Halococcus morrhuae* NRC16008 and *Haloferax mediterranei* ATCC33500. Nucleic Acids Res. **20:**3517.
19. **Krawiec, S., and M. Riley.** 1990. Organization of the bacterial chromosome. Microbiol. Rev. **54:**502–539.
20. **Lam, W. L., A. Cohen, D. Tsouluhas, and W. F. Doolittle.** 1990. Genes for tryptophan biosynthesis in the archaebacterium *Haloferax volcanii*. Proc. Natl. Acad. Sci. USA **87:**6614–6618.
21. **Lam, W. L., and W. F. Doolittle.** 1989. Shuttle vectors for the archaebacterium *Halobacterium volcanii*. Proc. Natl. Acad. Sci. USA **86:**5478–5482.
22. **Lam, W. L., S. M. Logan, and W. F. Doolittle.** 1992. Genes for tryptophan biosynthesis in the halophilic archaebacterium *Haloferax volcanii*: the *trpD-FEG* cluster. J. Bacteriol. **174:**1694–1697.
23. **Leffers, H., F. Gropp, F. Lottspeich, W. Zillig, and R. A. Garrett.** 1989. Sequence, organization, transcription and evolution of RNA polymerase subunit genes from the archaebacterial extreme halophiles *Halobacterium halobium* and *Halococcus morrhuae*. J. Mol. Biol. **206:**1–17.
24. **López-García, P., J. P. Abad, and R. Amils.** 1993. Genome analysis of different *Haloferax mediterranei* strains using pulsed-field gel electrophoresis. Syst. Appl. Microbiol. **16:**310–321.
25. **López-García, P., J. P. Abad, C. Smith, and R. Amils.** 1992. Genomic organization of the halophilic archaeon *Haloferax mediterranei*: physical map of the chromosome. Nucleic Acids Res. **20:**2459–2464.
26. **Ochman, H., and A. C. Wilson.** 1987. Evolution in bacteria: evidence for a universal substitution rate in cellular genomes. J. Mol. Evol. **26:**74–86.
27. **Pfeifer, F.** 1988. Genetics of halobacteria, p. 105–133. *In* F. Rodríguez-Valera (ed.), Halophilic bacteria, vol. II. CRC Press Inc., Boca Raton, Fla.
28. **Pfeifer, F., and M. Betlach.** 1985. Genome organization in *Halobacterium halobium*: a 70kb island of more (AT) rich DNA in the chromosome. Mol. Gen. Genet. **198:**449–455.
29. **Pfeifer, F., and U. Blaseio.** 1990. Transposition burst of the ISH27 insertion element family in *Halobacterium halobium*. Nucleic Acids Res. **18:**6921–6925.
30. **Pfeifer, F., U. Blaseio, and M. Horne.** 1989. Genome structure of *Halobacterium halobium*: plasmid dynamics in gas vacuole deficient mutants. Can. J. Microbiol. **35:**96–100.
31. **Pfeifer, F., K. Ebert, G. Weidinger, and W. Goebel.** 1982. Structure and functions of chromosomal and extrachromosomal DNA in halobacteria. Zentralbl. Bakteriol. Hyg. Abt. 1 Orig. C **3:**110–119.
32. **Pfeifer, F., G. Weidinger, and W. Goebel.** 1981. Genetic variability in *Halobacterium halobium*. J. Bacteriol. **145:**375–381.
33. **Riley, M., and K. E. Sanderson.** 1990. Comparative genetics of *Escherichia coli* and *Salmonella typhimurium*, p. 85–95. *In* K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington, D.C.
34. **Rosenshine, I., and M. Mevarech.** 1989. Isolation and partial characterization of plasmids found in three *Halobacterium volcanii* isolates. Can. J. Microbiol. **35:**92–95.
35. **Sapienza, C., and W. F. Doolittle.** 1982. Unusual physical organization of the *Halobacterium* genome. Nature (London) **295:**384–389.
36. **Sapienza, C., M. R. Rose, and W. F. Doolittle.** 1982. High-frequency genomic rearrangements involving archaebacterial repeat sequence elements. Nature (London) **299:**182–185.
37. **Schalkwyk, L. C., R. L. Charlebois, and W. F. Doolittle.** 1993. Insertion sequences on plasmid pHV1 of *Haloferax volcanii*. Can. J. Microbiol. **39:**201–206.
38. **Spiridonova, V. A., A. S. Akhmanova, V. K. Kagramanova, A. K. E. Köpke, and A. S. Mankin.** 1989. Ribosomal protein gene cluster of *Halobacterium halobium*: nucleotide sequence of the genes coding for S3 and L29 equivalent ribosomal proteins. Can. J. Microbiol. **35:**153–159.
39. **St. Jean, A., and R. L. Charlebois.** Unpublished data.
40. **St. Jean, A., B. A. Trieselmann, and R. L. Charlebois.** 1994. Physical map and set of overlapping cosmid clones representing the genome of the archaeon *Halobacterium* sp. GRB. Nucleic Acids Res. **22:**1476–1483.
41. **Torreblanca, M., F. Rodríguez-Valera, G. Juez, A. Ventosa, M. Kamekura, and M. Kates.** 1986. Classification of non-alkaliphilic halobacteria based on numerical taxonomy and polar lipid composition, and description of *Haloarcula* gen. nov. and *Haloferax* gen. nov. Syst. Appl. Microbiol. **8:**89–99.

41a. **Vettakkorumakankav, N. N.** Personal communication.

42. **Vettakkorumakankav, N. N., and K. J. Stevenson.** 1992. Dihydrolipoamide dehydrogenase from *Haloferax volcanii*: gene cloning, complete primary structure, and comparison to other dihydrolipoamide dehydrogenases. Biochem. Cell Biol. **70:**656–663.
43. **Weidinger, G., G. Klotz, and W. Goebel.** 1979. A large plasmid from *Halobacterium halobium* carrying genetic information for gas vacuole formation. Plasmid **2:**377–386.