

# Haloarchaeal Megaplasמידs

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**Abstract** Many salt-loving archaea, or haloarchaea, commonly harbor one or more megaplasמידs in their genomes. The haloarchaeal model organism, *Halobacterium* sp. NRC-1, contains two related replicons, pNRC100 (191 kb in size) and pNRC200 (365 kb), both of which code for buoyant gas vesicles as well as other genes that are

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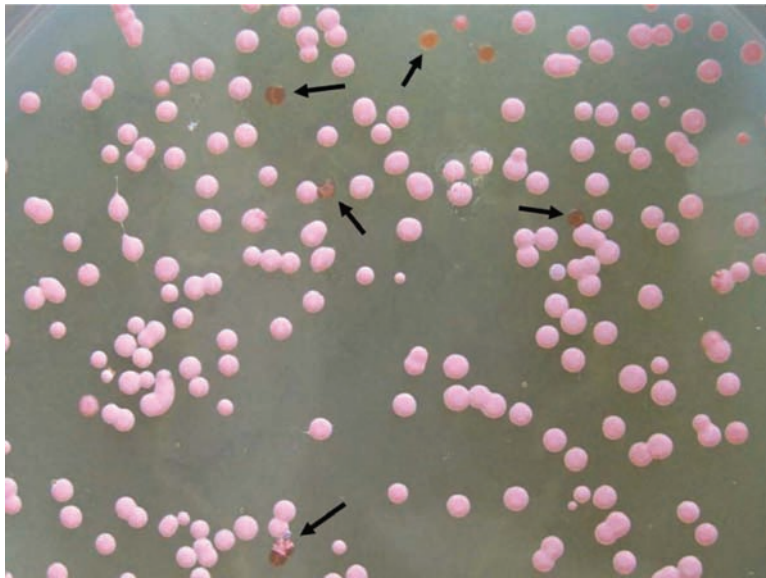
important or essential for the host. Two other haloarchaea, *Haloarcula marismortui* and *Haloferax volcanii*, with larger genomes have a more complex complement of extrachromosomal replicons, eight in the former, including four megaplasms, and four in the latter, including two megaplasms and one very large plasmid. Two other sequenced haloarchaea, *Natronomonas pharaonis* and *Haloquadratum walsbyi*, have fewer and smaller extrachromosomal replicons, with a single megaplasmid in the former and only a relatively small plasmid in the latter. We review the current state of knowledge on these sequenced megaplasms, including the eukaryotic type replication protein genes present in most, IS elements populating many, and unusual (e.g., gas vesicle), important (cytochrome oxidase and thioredoxin/thioredoxin reductase), and even essential (transcription and replication factors and an aminoacyl-tRNA synthetase) genes on several of these large replicons. The role of megaplasms, some of which may qualify as small chromosomes (minichromosomes), in the evolution of haloarchaeal genome architecture is discussed.

## 1 Introduction

The advent of high-throughput mapping and sequencing technologies developed in the 1980s and 1990s and subsequent post-genomic methodologies of the early twenty-first century have allowed detailed understanding of prokaryotic genomes, including members of the third domain of life, the archaea (Fraser et al. 2004). Archaeal genomes range in size from 0.5–5.75 Mbp and are organized, like most bacterial genomes, with a single main circular chromosome, and on occasion, with accessory plasmids or extrachromosomal elements. Among the halophilic or salt-loving Archaea (also called haloarchaea), which are geographically widely distributed in salty environments around the world, large plasmids are very common. Nine of these haloarchaeal megaplasms have been completely sequenced and several have been classified as minichromosomes due to the presence of important or essential genes (DasSarma 2004). The best studied example, pNRC100, in the model *Halobacterium* sp. NRC-1, is distinguished by an abundance of transposable IS elements that promote DNA rearrangements and cause frequent phenotypic variability (Fig. 1) (DasSarma and Arora 1997; Ng et al. 1998).

Currently, of the five complete haloarchaeal genome sequences (Tables 1 and 2), four harbor megaplasms larger than 100 kb. The genome of the first sequenced haloarchaeon, *Halobacterium* sp. NRC-1, 2.57 Mbp in size, contains two large, related megaplasms, pNRC100 and pNRC200, specifying buoyant gas vesicles (Ng et al. 1998; Ng et al. 2000; DasSarma 2004). Because of the presence of many important and a few essential genes, these replicons were proposed to be essential minichromosomes. *Halobacterium* is widely distributed in nature, for example, as a major component of Great Salt Lake in the western USA (Baxter et al. 2005), and is one of the most intensively studied archaea to date (DasSarma et al. 2006). Two halophiles from the Dead Sea, *Haloferax volcanii* and *Haloarcula marismortui*, have bigger, more complex genomes with larger numbers of megaplasms (Charlebois et al. 1991; Baliga et al. 2004). *H. marismortui* has a 4.3 Mbp genome

with four megaplasms ranging in size from 411 to 133 kb (pNG700, pNG600, and pNG500, and one which was designated as Chromosome II). *H. volcanii* contains a slightly smaller genome, 4.1 Mbp, with two megaplasms, pHV4 (636 kb) and pHV3 (438 kb), which are the largest characterized among haloarchaea to date. In addition, *H. volcanii* contains a third large plasmid, pHV1 (85 kb), which is



**Fig. 1** Colonies of *Halobacterium* sp. NRC-1 on a salt agar plate showing wild-type (pink, opaque) gas vesicle-containing colonies, and with arrows, mutant (orange, translucent, or sectorized) gas vesicle mutants. Mutants result from high-frequency DNA rearrangements in megaplasms pNRC100 and pNRC200

**Table 1** Sequenced haloarchaeal organisms

Organism	Physiology and ecology	Genome	Reference
<i>Halobacterium</i> sp. NRC-1 ATCC 700922, JCM 11081	Extreme halophile, facultative anaerobe, and phototroph	2.6 Mbp, 2 megaplasms	DasSarma (2004)
<i>Haloferax volcanii</i> DS2 ATCC 29605, DSM 3757, JCM 8879	Moderate halophile, prototroph, from Dead Sea mud	4.1 Mbp, 2 megaplasms	Charlebois et al. (1991)
<i>Haloarcula marismortui</i> ATCC 43049, DSM 3752; JCM 8966	Extreme halophile, metabolically versa- tile, from Dead Sea	4.3 Mbp, 4 megaplasms	Baliga et al. (2004)
<i>Natronomonas pharaonis</i> strain Gabara ATCC 35678, DSM 2160, JCM 8858	Alkaliphilic extreme halophile from soda lake	2.8 Mbp, 1 megaplasmid	Falb et al. (2005)
<i>Haloquadratum walsbyi</i> HBSQ001, DSM16790, JCM 12895	Square-shaped extreme halophile from solar saltern	3.2 Mbp, no megaplasmid	Bolhuis et al. (2006)



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