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SHORT COMMUNICATION

Lateral gene transfer occurring in haloarchaea: an interpretative imitation study

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Abstract Lateral gene transfer (LGT) plays an important role in the molecular evolution of haloarchaea. Polyethylene glycol-mediated LGT in haloarchaea has been demonstrated in the laboratory, yet few explanations have been put forward for the apparently common, natural occurrence of plentiful plasmids within haloarchaeal cells. In this study, LGT was induced in two genera of haloarchaea, *Haloferax* and *Halorubrum*, by modification of salt concentration of media—a factor that may vary naturally in native haloarchaeal habitat. Minimal growth salt concentrations (MGSCs) of four strains of haloarchaea from these two genera were established, and transformations using two circular double-stranded DNAs (dsDNAs), pSY1 and pWL102, were then produced in media at strain-appropriate MGSCs. The four strains of haloarchaea were transformed successfully by both kinds of dsDNAs with an efficiency of 10^2–10^3 transformants per microgram dsDNA. The transformation under reduced salt concentration may be an imitation of natural LGT of dsDNA into haloarchaea when salinity in normally hypersaline environments is altered by sudden introduction of fresh water—for example, by rainfall, snow-melt, or flooding—providing a reasonable interpretation for haloarchaea being naturally richer in plasmids than any other known organisms.

Keywords Horizontal gene transfer · Evolution · Cell permeability · dsDNA · Transformation

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Introduction

Lateral (horizontal) gene transfer (LGT) plays an important role in molecular evolution of microorganisms (Garcia-Vallvé et al. 2000; Rhodes et al. 2011). Archaea are microorganisms that were originally discovered in extreme environments. Haloarchaea were discovered in hypersaline environments (HSE), in which until recently they were thought to occur only (Rosenshine et al. 1989; Elshahed et al. 2004; Purdy et al. 2004; Soppa 2006; Fukushima et al. 2007; Oxley et al. 2010). High salinities can preserve even naked DNA for exceptionally long periods of time (Rhodes et al. 2011). Bath et al. (2006) and Roine et al. (2010) isolated and identified two kinds of circular dsDNAs from HSE, suggesting that circular dsDNAs remain stable in HSE (Rhodes et al. 2011). Furthermore, Sun et al. (2006) and Zhou et al. (2007) reported that haloarchaea are rich in plasmids; and plasmids represent more than 25 % of the genetic material of the cells in some haloarchaeal strains (Holmes et al. 1995).

During the past few years, a variety of plasmids have been separated from haloarchaeal cells (Ye et al. 2003; Zhou et al. 2004, 2007; Sun et al. 2006).

LGT events are generally identified using phylogenetic and compositional methods (Ragan et al. 2006). Rhodes et al. (2011) compared LGT events in haloarchaea and Thermoprotei, and found over 1,000 events in haloarchaea. However, how the extra-cellar DNA in the hypersaline environment enters the haloarcheal cell remains unclear. Cline and Doolittle (1987) developed an in vitro DNA transformation approach with the high efficiency in haloarchaea in the presence of polyethylene glycol (PEG). It is well known that PEG can promote cell permeability (Yanakawa et al. 1985; Zheng et al. 2005). However, PEG is very rare in HSE if it is ever present, and that leads to a critical question: how can circular dsDNAs in an HSE enter haloarchaeal cells without PEG mediation?

Unlike other archaea, haloarchaea have a cell membrane with a structure that is influenced by salt concentrations (LoBasso et al. 2008). The question arises as to whether a change in salt concentration can change cell wall permeability, especially with regard to circular dsDNAs. To test whether alteration of permeability does occur and, if it does, whether LGT in haloarchaea can be mediated by altering salt concentration, the following experiment was devised.

Minimal growth salt concentrations (MGSCs) for genera *Haloferax* (hereafter abbreviated as *Hfx.*) and *Halorubrum* (hereafter abbreviated as *Hrr.*) were established. A lower osmotic pressure condition was established near the MGSC of each strain, and the haloarchaeal cells were treated at that lower osmotic pressure by the introduction of plasmids pSY1 and pWL102, which were intended to serve as two different types of selective markers should transformation occur. After establishment of cultures of the treated haloarchaea, experiments were carried out to test whether transformation had occurred and, if so, at what efficiency.

Materials and methods

Strains and growth conditions

The strains of *Escherichia coli* DH5α, haloarchaea and plasmids used in this study are listed in Table 1. All the strains of haloarchaea were grown in modified growth medium (MGM), comprising per liter of solution: 1 g of yeast extract (Difco Laboratories), 5 g of peptone (Oxoid), 144 g of sodium chloride (NaCl), 18 g of $MgCl_2{\cdot}6H_2O$, 21 g of $MgSO_4{\cdot}7H_2O$, 4.2 g of KCl, 0.5 g of $CaCl_2{\cdot}2H_2O$, 0.12 g of $NaHCO_3$ and 0.48 g of NaBr (Nuttall and Dyall-Smith 1993). In order to detect the concentration of NaCl required for the MGSC of the halophiles, NaCl concentration was adjusted incrementally from 0.6 to 1.8 mol/l, with other components constant. Selective agar plates were made by adding novobiocin (Sigma) (4 μg/ml) or mevinolin (Sigma) (dissolved with 100 % ethanol at 5 μg/ml). *E. coli* strain DH5α with plasmid pSY1 or pWL102 were grown at 37 °C in Luria–Bertani (LB) medium (Sambrook and Russell 2001), containing ampicillin at 100 μg/ml, when necessary, for propagating plasmids. Agar plates were made by adding 1.5 % (w/v) agar powder to liquid media.

Transformation procedure

All plasmids were extracted and purified before being added to suspended cells as suggested by Sambrook and Russell (2001). Four halophilic strains served as hosts: *Hfx. volcanii* DS70, *Hfx.* sp. H4, *Hrr.* sp. YYJ and *Hrr.* sp. CY. When each strain was cultured to mid-logarithmic phase, and the optical density (OD) at 600 nm reached 0.8, 400 μl samples of each culture were placed in tubes, and cells were harvested by centrifuging at 10,000 r/min for 5 min. Supernatants were discarded, and cells were resuspended with 400 μl, 0.8 and 1.6 mol/l NaCl solution for genera *Hfx.* and *Hrr.* respectively, treated by addition of 20 μl (about 2 μg) circular dsDNAs, and then statically cultured at room temperature. One hour later, all the suspensions

Table 1 Plasmids and strains used in this study

Plasmids and strains	Description	Source
pSY1	Amp^r/Nov^r, shuttle vector, ori pHK2 and pSK(+)	Yang et al. (2003)
pWL102	Amp^r/Mev^r, ori shuttle vector pHV2 and pBR322	Han et al. (2007)
E. coli DH5α	*Sup*E delta *lac*U169 *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *rel*A1 *thi*-1	This study
Haloferax volcanii DS70	Halophile without plasmid	Allers (2010)
Haloferax sp. H4	Halophile without plasmid	This study
Halorubrum sp. YYJ	Halophile without plasmid	This study
Halorubrum sp. CY	Halophile without plasmid	This study

were spread on the selective agar plates, with 210 µl on each plate. The plates were then cultured at 37 °C for 2 weeks. The plates were treated with antibiotics: novobiocin (4 µg/ml) for pSY1 and mevinolin (5 µg/ml) for pWL102. In controls, the plasmid DNA was replaced by 20 µl ddH_2O. On other hand, *Hfx. volcanii* DS70 and plasmid pSY1 were taken for instance to determine the influence of NaCl concentration on transformation efficiency. The gradient of NaCl concentration was adjusted incrementally 0.3 to 2.4 mol/l. Other procedures remain unchanged.

Extraction of circular dsDNA

The transformants on MGM selective plates were picked up with toothpicks, and inoculated into liquid MGM medium containing appropriate antibiotics (4 µg/ml novobiocin for pSY1 or 5 µg/ml mevinolin for pWL102). For each of the four host strains, five transformants were taken from randomly selected novobiocin agar plates and six transformants were taken from randomly selected mevinolin agar plates. Plasmids were extracted by the method of alkaline lysis and detected by electrophoresis (Sambrook and Russell 2001).

Restriction endonuclease digestion

In order to determine whether the plasmids extracted from the transformants were identical to the plasmids added to the cell suspensions, the following method was used: all proposed plasmids were digested with *Nco*I (TaKaRa), following the manufacturer's instructions, detected by electrophoresis, and then visually compared with original plasmid dsDNA.

Efficiency of transformation

Plasmids were dissolved in ddH_2O. The concentration and purity were determined by ultra violet (UV) spectrophotometer at the OD at 260 and 280 nm. Sterile distilled water was used as a blank. The number of transformants on selective agar plates was calculated. The efficiency of transformation was defined to be the number of transformants (colonies) produced by each microgram of dsDNA.

GenBank accession numbers

The nucleotide sequences of the 16S rRNA gene of *Hfx.* sp. H4, *Hrr.* sp. YYJ, and *Hrr.* sp. CY were deposited in

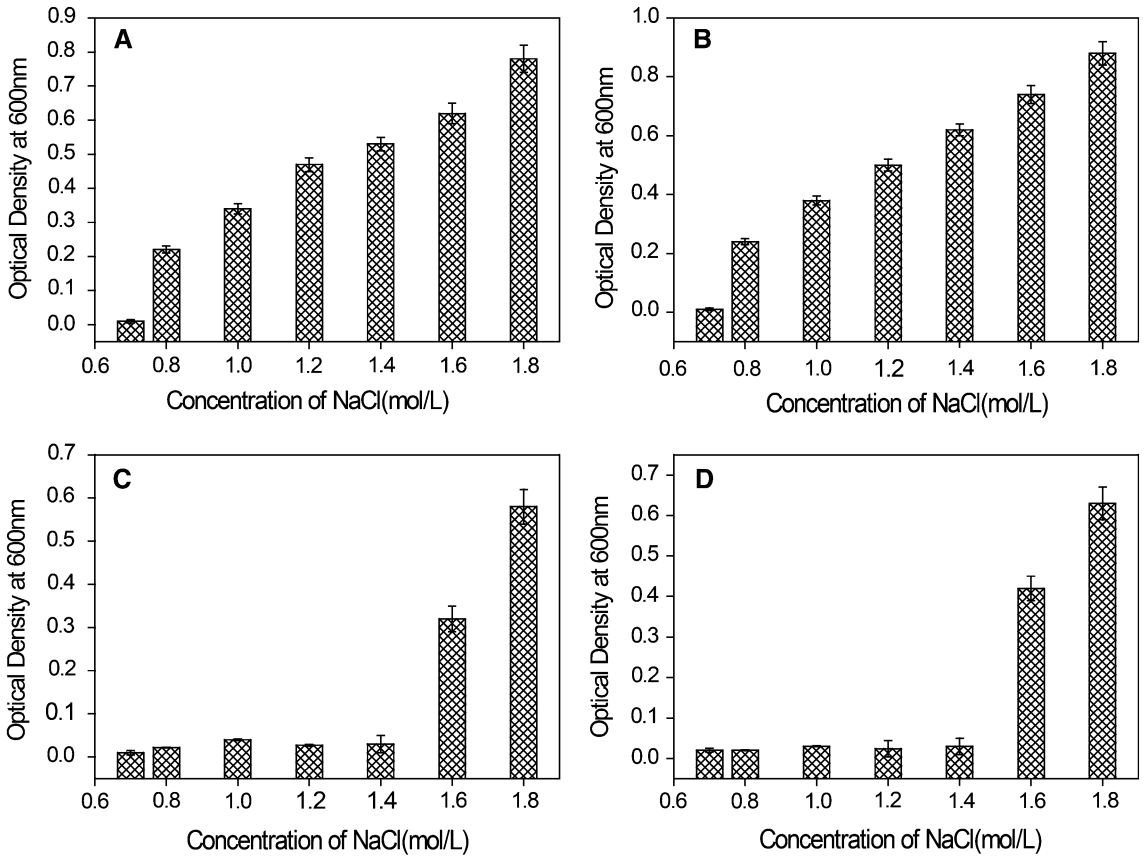

Fig. 1 Determination of MGSC. **a** *Haloferax volcanii* DS70. **b** *Haloferax* sp. H4. **c** *Halorubrum* sp. YYJ. **d** *Halorubrum* sp. CY

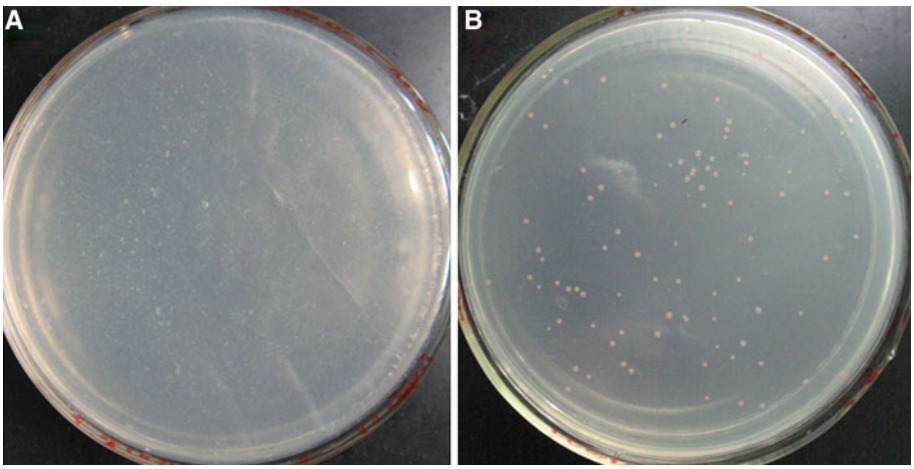

Fig. 2 Transformants on selective agar plates. All the host strains used in transformation were plasmid-free. The DS70 strain of *H. volcanii* was chosen as representative, others were omitted. Plate **a** is blank. Transformants were present on plate **b**

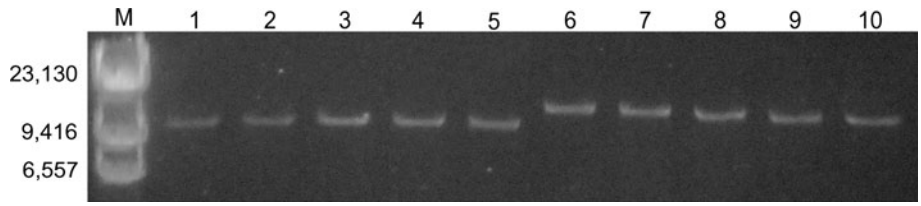

Fig. 3 Determination of plasmids. Plasmids extracted from the four transformed strains of *Haloferax* and *Halorubrum* and the standard plasmids were digested with *Nco*I. Original plasmids occupied lanes as follows: pWL102 (*lane 1*), pSY1 (*lane 6*). Plasmids extracted from pWL102 transformed strains occupied lanes as follows: *Hfx. volcanii* DS70 (*lane 2*), *Hfx.* sp. H4 (*lane 3*), *Hrr.* sp. YYJ (*lane 4*), and *Hrr.* sp. CY (*lane 5*). They were identical to standard plasmid pWL102 (*lane 1*) on size and DNA band location. Plasmids extracted from pSY1 transformed strains occupied lanes as follows: *Hfx. volcanii* DS70 (*lane 7*), *Hfx.* sp. H4 (*lane 8*), *Hrr.* sp. YYJ (*lane 9*), *Hrr.* sp. CY (*lane 10*). They were identical to standard plasmid pSY1 (*lane 6*) on size and DNA band location. M, λ DNA/*Hind*III DNA marker

GenBank with accession numbers FJ746722, FJ771026, and FJ267614, respectively.

Results and discussion

Minimal growth salt concentration (MGSC) of haloarchaeal cells from the genera *Hfx*. and *Hrr*. was determined based on the universal MGM medium developed by Nuttall and Dyall-Smith (1993). It was found that the MGSC of *Hfx. volcanii* DS70, *Hfx.* sp. H4, *Hrr.* sp.YYJ, and *Hrr.* sp. CY were 0.8, 0.8, 1.6, and 1.6 mol/l, respectively (Fig. 1a–d).

By employing the newly determined MGSC (Fig. 1), we simplified the universal transformation procedure (Cline and Doolittle 1987) of haloarchaea by omitting three steps, and substituted ddH_2O for PEG. When mixed with plasmid pSY1 or pWL102, the strains of both genera *Hfx*. and *Hrr*. formed single colonies on MGM selective plates under minimal growth conditions (Fig. 2b). No such colonies were formed in the control (Fig. 2a). The controls were plasmid-free. The results show that *Hfx. volcanii* DS70, *Hfx.* sp. H4, *Hrr.* sp. YYJ and *Hrr.* sp. CY were transformed. Transformants were obtained on selective agar plates (Fig. 2), and determined by the digestion of

Table 2 Transformation efficiency

Strains	Plasmids	
	pSY1	pWL102
	Transformation efficiency (transformants/μg dsDNA) ($\times 10^3$)	
H. volcanii DS70	1.60	0.90
Haloferax sp. H4	1.25	0.55
Halorubrum sp. YYJ	0.02	0.01
Halorubrum sp. CY	0.01	0.01

restriction enzyme *Nco*I (Fig. 3). The transformation efficiency of the genus *Hfx*. was about 10^3 transformants per microgram of circular dsDNA, while 10^2 or less for the genus *Hrr*. (Table 2). From the best fit-model of gauss, the transformation efficiency of *Hfx. volocanii* and pSY1 reached its maximum when the NaCl concentration at the relevant MGSC and decreased rapidly with either increase or decrease of NaCl concentration (Fig. 4). There is no doubt that NaCl concentration plays a critical role on transformation efficiency of haloarchaeal cells.

Although the transformation efficiencies obtained with our method are lower than those reported (e.g., 10^3–10^6) by Cline and Doolittle (1987), the process that we utilized is

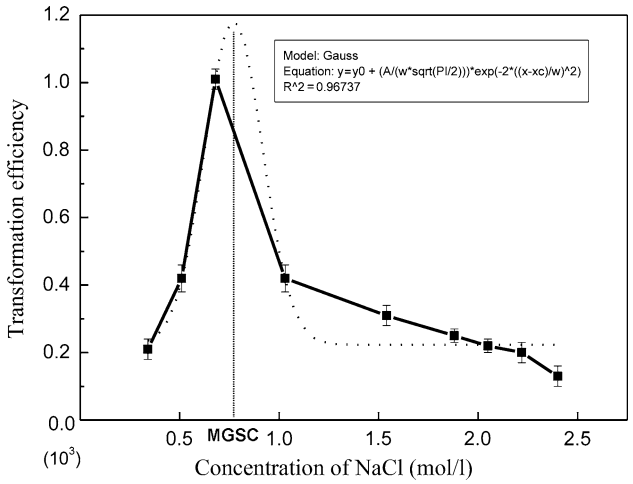

Fig. 4 Influence of NaCl concentration on transformation efficiency. *Hfx. volcanii* DS70 was treated with a gradient of NaCl concentration, from 0.3 to 2.4 mol/l, and transformed with pSY1. *Dotted line* gauss fit of the data. *MGSC* minimal growth salt concentration

more likely to model actual behavior in nature. In inducing transformation the only variable altered by us was salt concentration. Successful PEG-induced transformations are attributed to a PEG-induced increase in cell permeability in yeast (Yanakawa et al. 1985; Zheng et al. 2005). It seems that it is reasonable to use the same terms in the present case-local changes in habitat salt concentration (e.g., to near a relevant MGSC) that induce transformation and, hence, must have induced increased cell permeability in the haloarchaea (Fig. 4). Since our experimental procedure imitates natural introduction of fresh water to a high salinity habitat, we hypothesize that LGT occurred after the extracellar dsDNAs enter into haloarchaeal cell, when high salt concentration in a local region containing haloarchaea drops dramatically in a short time due to sudden availability of fresh or low-salinity water by such means as rain, snow-melt, or flooding of a fresh water stream or low salinity salt water brought inland by a storm surge (Fig. 5). Under such circumstances, circular dsDNA (virus or

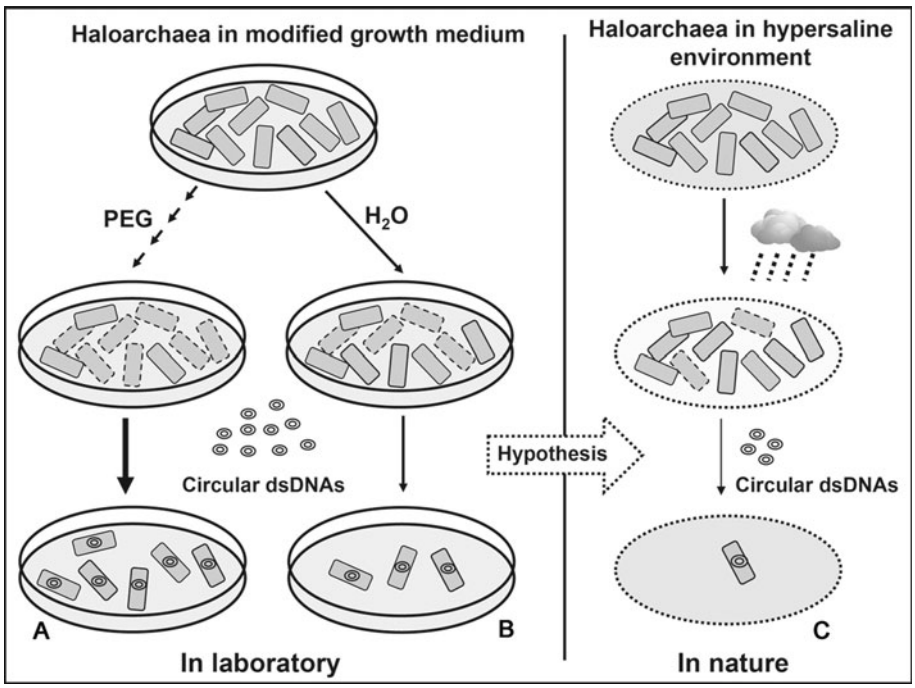

Fig. 5 Schemata showing hypothetical HGT in haloarchaea in nature as suggested by two methods for inducing HGT in the laboratory. Two artificial transformation procedures are shown on the *left*, and a hypothesis for a possible natural gene transfer mechanism is shown on the *right*. The universal transformation procedure based on PEG mediation is depicted by schema *A*. PEG has been widely used to promote cell permeability. We observed that when haloarchaea experience relatively low salinity, their cell membrane also is permeable by dsDNAs—the effect of ddH_2O was equal to PEG mediation to some extent. Transformation based on lowering salinity is depicted by schema *B*. Although, there was no PEG in reported HSEs, haloarchaeal cells were richer in plasmids. *Dark-colored*, *solid-outline rectangles* represent haloarchaea that have not been treated. *Light-colored*, *solid-outline rectangles* represent intact haloarchaea. *Dotted-outline rectangles* represent competent cells. Treatment is schematically indicated by *clusters* of the *double-stranded ring*, representing *circular* dsDNA. At the bottom of each schema, results of transformation are depicted; in comparing any two schemata, the ratio of the counts of *rectangles* including *circular* dsDNA approximates the ratio of transformation efficiencies of the processes represented by the schemata

plasmids) in natural HSE (Bath et al. 2006; Mei et al. 2007; Roine et al. 2010) may gain access to haloarchaea in nature (Fig. 5). In addition, Rhodes et al. (2011) identified 1,226 putative LGT events in haloarchaea on the basis of genomic database. Our hypothesis may serve as a partial interpretation for the observation that numerous LGT events have naturally occurred in haloarchaea, and that haloarchaeal cells are richer in plasmids than those of any other known organisms (Gutiérrez et al. 1986; Sun et al. 2006; Zhou et al. 2007).

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