REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	20 Oct 97	Final Report 1 Apr 94 - 31 Aug 97

4. TITLE AND SUBTITLE
Genetic exchange between mutant strains of Sulfolobus acidocaldarius: Analysis, applications, and significance for hyperthermophiles

5. FUNDING NUMBERS
N0014-94-1-0393

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

A prokaryotic micro-organism originally isolated from terrestrial hot springs, Sulfolobus acidocaldarius, was studied for its ability to exchange and recombine segments of its chromosome. Mutant strains were isolated and used to quantitatively assay this process. The genetic exchange was found to be a form of conjugation that differs from bacterial conjugation with respect to the symmetry of DNA transfer and other properties. Other fundamental genetic phenomena of prokaryotes from geothermal habitats were studied for the first time using S. acidocaldarius; these included photoreactivation, UV-induced mutagenesis, and stimulation of genetic exchange by UV. The rate of spontaneous mutation was measured at 75 degrees C in S. acidocaldarius and was found to be nearly the same as that of the bacterium Escherichia coli at 37 degrees C. This provided the first evidence that the archaea which populate geothermal habitats can maintain genetic fidelity even at extremely high temperatures The basic information gained in these studies portrays the microbial inhabitants of thermal environments in a new light, suggesting that they may mutate infrequently in nature but may exchange genes at a signficant rate.

14. SUBJECT TERMS
Archaea; geothermal habitats; mutant strains; genetic exchange; cell envelope; spontaneous mutation

15. NUMBER OF PAGES
4

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
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FINAL REPORT

Grant# N0014-94-1-0393 R&T Code: 341222---01

PRINCIPLE INVESTIGATOR: Dennis W. Grogan, Ph.D.

INSTITUTION: University of Cincinnati

GRANT TITLE: Genetic exchange between mutant strains of *Sulfolobus acidocaldarius*: Analysis, applications, and significance for hyperthermophiles

AWARD PERIOD: 1 April 1994 - 31 August 1997

OBJECTIVE: To study natural genetic exchange in an Archaeon from geothermal environments, as a means of i) elucidating molecular aspects of cellular function under extreme conditions, ii) developing genetic techniques for extremophiles, and iii) gaining insights into the genetic dynamics of hyperthermophilic communities in nature.

APPROACH: The formation of genetically recombinant strains of *Sulfolobus acidocaldarius* is being characterized by a variety of genetic and physiological criteria.

ACCOMPLISHMENTS: We have documented the first example of chromosomal exchange in an extremely thermophilic archaeon. The process appears to be a form of conjugation, since it occus only when two genetically distinct and living cells are allowed to come into contact. All sufficiently stable *S. acidocaldarius* mutants have been found to form recombinants, as measured by genetic assay. Recombinants from a genetic cross can also exchange markers with either parent. These observations, combined with the absence of any known extrachromosomal elements in *S. acidocaldarius*, suggest that genetic exchange is a natural, intrinsic property of this organism.

The PI investigated the biochemical architecture of the *S. acidocaldarius* cell surface, since it presumably plays a major role in the cell-cell interactions which lead to conjugation. The results showed that the S-layer of *S. acidocaldarius*, which in other laboratories has been extensively studied by EM and used to fabricate nanometer-scale patterns on silicon and other substrates, consists of two biochemically distinct glycoprotein species. The larger, more abundant subunit appears to form the paracrystalline surface layer, whereas the smaller subunit anchors this layer to the cytoplasmic membrane. Several new glycoproteins of *S. acidocaldarius* were identified as non-

sacculus membrane proteins, and could be specifically depleted in whole cells, either by protease treatment or by growth in the presence of the glycosyltransferase inhibitor tunicamycin.

In the course of our research, we observed that UV-inactivated cells of *S. acidocaldarius* rapidly regain viability when exposed to white light. This 'photoreactivation' is known in other organisms and results from the splitting of pyrimidine dimers in chromosomal DNA. Photoreactivation was strictly dependent upon illumination with visible light and was not attenuated by prior dark-incubation. The rates determined at several temperatures and at several wavelengths of light provide evidence for a DNA photolyase having a broad action spectrum.

Photoreactivation was also useful as an experimental tool for investigating DNA damage and repair in archaea from geothermal environments. We used auxotrophic mutants of *S. acidocaldarius* to assess genetic and physiological effects short-wavelength UV light, i.e., killing, phenotypic reversion, and the formation of genetic recombinants. The corresponding *Escherichia coli* auxotrophs served as controls. The results showed *S. acidocaldarius* to be about twice as UV-sensitive as *E. coli* and to be equally UV-mutable on a surviving-cell basis. Furthermore, UV irradiation significantly increased the frequency of recombinants recovered from genetic-exchange assays of *S. acidocaldarius*. The observed UV effects were due to the short-wavelength (i.e., UV-C) portion of the spectrum and were effectively reversed by visible light (photoreactivation). Thus, the observed responses are probably initiated by the formation of pyrimidine dimers in the *S. acidocaldarius* chromosome.

In addition to damage from radiation, archaea growing in geothermal environments are presumably subjected to spontaneous decomposition of DNA accelerated by their extremely high growth temperatures. To investigate the extent to which the cells compensate for this, we developed a quantitative assay of spontaneous loss-of-function mutation in *S. acidocaldarius*, based on the selection of mutants resistant to 5-fluoro-orotic acid. Maximum-likelihood analysis of mutant distributions in wild-type cultures yielded estimates of $2.8 \pm 0.8 \times 10^{-7}$ and $1.4 \pm 0.6 \times 10^{-7}$ mutational events per cell per division cycle for the *pyrE* and *pyrF* loci, respectively. The corresponding rates were somewhat higher in two amino-acid auxotrophs of *S. acidocaldarius* and somewhat lower in a pyrimidine auxotroph. Growth at different temperatures yielded variations of similar magnitude having no clear-cut pattern, whereas other physiological stresses had no measurable effects. The average rate of forward mutation at the *pyrE* and *pyrF* loci in wild-type *S. acidocaldarius* is very close to the average rate reported for *his* genes of *Escherichia coli*.

This mutation rate is about 10^{-7} times the predicted rate of DNA depurination at temperature and pH of the *S. acidocaldarius* cytoplasm during growth.

Finally, we have studied various parameters of genetic exchange in order to clarify its possible mechanism and experimental usefulness. The efficiency of genetic exchange was essentially independent of the density of cells deposited on the surface of solid media. Furthermore, recombinants were formed in liquid suspensions at 22 °C, as indicated by high recombinant frequencies resulting from mixtures plated at low cell densities, or in liquid suspensions that were never plated. The apparent initiation of genetic exchange at 22 °C was resistant to DNase, prior digestion of parental cells with protease from *Streptomyces griseus*, and all other chemical agents tested. The results support prior indications that chromosomal marker exchange in *S acidocaldarius* proceeds via conjugation, and further indicate that conjugation can initiate quickly in dilute liquid suspension at room temperature. This latter property distinguishes the mating system of *S. acidocaldarius* from that of *Haloferax volcanii* but is consistent with conjugational transfer of plasmid pNOB8 among other *Sulfolobus* spp. The number of *S. acidocaldarius* recombinants formed in our assays (10^{-4} to 10^{-5} per c.f.u.) greatly exceeds the number of spontaneous forward mutational events per generation for two biosynthetic genes.

CONCLUSIONS: The genetic exchange process of *S. acidocaldarius* differs from conventional bacterial conjugation and provides a new tool for genetic manipulation of thermophilic archaea in the laboratory. New insight into the structure and function of the cell envelope of *S. acidocaldarus* cell may help future studies elucidate this process in mechanistic terms. Photoreactivation in *S. acidocaldarius* represents the first DNA repair process to be measured in an archaeon from geothermal environments, and the dose-response relationships of UV effects provide the first evidence of error-prone DNA repair and genetic recombination induced by DNA damage in hyperthermophiles. Accurate measurements of spontaneous mutation rates provide the first experimental estimates of the genetic fidelity maintained by archaea that populate geothermal environments. These rates are low, implying that this hyperthermophile has cellular mechanisms, yet unidentified, for avoiding and repairing high levels of DNA damage at extremely high temperatures. The fact that recombinants at particular genetic loci seem to occur more frequently than spontaneous mutations at the same loci suggests that genetic exchange may be the dominant force in the genetic dynamics of natural *Sulfolobus* populations.

SIGNIFICANCE: Considerable progress is being made toward understanding chromosomal genetic exchange in *S. acidocaldarius*. This basic genetic process has fundamental implications for gene flow and evolutionary dynamics in extreme environments. It also provides an experimental tool, which, in combination with other genetic manipulations developed during this project, provide a context for investigating DNA damage and repair for the first time in archaea from geothermal environments.

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