ISSN : 1225-9918
Journal of Life Science **2011** Vol. 21. No. 10. 1370~1376 DOI : http://dx.doi.org/10.5352/JLS.2011.21.10.1370

Isolation and Characterization of Pyrimidine Auxotrophs from the Hyperthermophilic Archaeon *Sulfolobus acidocaldarius* DSM 639

Kyoung-Hwa Choi and Jaeho Cha*

Department of Microbiology, Pusan National University, Busan 609-735, Korea

Received September 16, 2011 / Revised September 22, 2011 / Accepted September 22, 2011

To study the functional genomic analysis of a crenachaeon *Sulfolobus acidocaldarius*, we have constructed an auxotrophic mutant based on *pyrEF*, which encodes the pyrimidine biosynthetic enzymes orotate phosphoribosyltransferase and orotidine-5'-monophosphate decarboxylase. *S. acidocaldarius* was shown to be sensitive to 5-fluoroorotic acid (5-FOA), which can be selected for mutations in *pyrEF* genes within a pyrimidine biosynthesis cluster. Spontaneous 5-FOA-resistant mutants by ultraviolet, KH1U and KH2U, were found to contain two point mutations and a frame shift mutation in *pyrE*, respectively. Mutations at these sites from KH1U and KH2U decreased the activity of orotate phosphoribosyltransferase encoded by the *pyrE* gene and blocked the degradation of 5-FOA into toxic 5-FOMP and 5-FUMP that kill the cells. Therefore, KH1U and KH2U were uracil auxotrophs. Transformation of *Sulfolobus-Escherichia coli* shuttle vector pC bearing *pyrEF* genes from *S. solfataricus* P2 into *S. acidocaldarius* mutant KH2U restored 5-FOA sensitivity and overcame the uracil auxotrophy. This study establishes an efficient genetic strategy towards the systematic knockout of genes in *S. acidocaldarius*.

Key words : Pyrimidine biosynthesis, *Sulfolobus acidocaldarius*, uracil auxotroph, 5-fluoroorotic acid

Introduction

Sulfolobus species, the first isolated organisms from hot spring in Yellowstone National Park, USA, are one of the best studied model organisms of archaea. It grows optimally at 77℃ and pH 3 under aerobic and heterotrophic conditions. Many studies of the replication [3], transcription [13-15], and translation [11,18,24] in archaea have been carried out, but *in vivo* experiments were for a long time hampered by the lack of appropriate genetic system [1,20,22]. Nevertheless, various genetic tools and methods have been reported especially in halophilic archaea [7,19]. For *Sulfolobus* species, several genetic systems such as high efficiency transformation technique, construction of shuttle vector, and isolation of gene disruption mutant have been published in the past 10 years, but it remains many problems to overcome the genetic studies of *Sulfolobus* [4,5,10].

Of that, the maintenance of plasmid against restriction-modification system of *Sulfolobus* and post-treatment following electroporation were crucial for successful transformation of *Sulfolobus*. Furthermore, the construction of suitable mutants for genetic studies has also been hampered from high background growth and spontaneous reversion frequencies. A gene disruption system in *S. solfataricus* P2 has been developed by P. Blum and coworkers [23]. In this system, *lacS* gene was used as a counterselectable marker. A target gene inserted with *lacS* gene was integrated into the host chromosome by a homologous recombination in order to replace the target gene to be deleted. Successful mutants were selected for a lactose medium as sole carbon and energy source. Little studies about disruption of targeted genes for *S. acidocaldarius* have been published [12]. Suitable conditions and techniques for transformation into *S. acidocaldarius* have been reported [16]. However, no detailed studies on metabolism such as sugar utilization by disrupted mutant have yet been published.

For genetic experiment in hyperthermophilic crenarchaea such as *Sulfolobus*, antibiotic selection is not as effective as euryarchaea *Thermococcus*. Only two *Sulfolobus-E. coli* shuttle vectors, namely pAG21 and pEXSs, have been described so far [2,9]. An antibiotics simvastatin which worked efficiently in *Thermococcus* was easily degraded during the high temperature culture of *S. acidocaldarius*. It is probably thought that most antibiotics have low stability under high temperatures. In order to overcome these problems, uracil auxotrophic mutant has been used for genetic studies in *Sulfolobus*. Uracil auxotrophic mutant has 5-FOA (5-fluoroorotic acid) resistance by mutation within *pyrE* or *pyrF* gene. Orotate phosphoribosyltransferase and or-

***Corresponding author**
Tel : +82-51-510-2196, Fax : +82-51-514-1778
E-mail : jhcha@pusan.ac.kr

otidine-5′-monophosphate decarboxylase encoded by these genes are key enzymes which catalyze the last steps in pyrimidine biosynthesis (Fig. 1). These enzymes convert 5-FOA to toxic metabolites and growth of cells by these is inhibited. For uracil auxotroph mutant, it was not able to convert 5-FOA to toxic substrates because of mutation of *pyrE* or *pyrF* gene and thus it could be grown at high concentration of 5-FOA. Therefore, RNA and DNA are synthesized via conversion of uracil to UMP in *pyrEF* mutant.

In this study, uracil auxotrophic mutant with low reversion frequencies was constructed for genetic studies in *S. acidocaldarius*. Spontaneous mutation by UV irradiation was performed to obtain *pyrE*-deficient mutant KH1U and KH2U. We demonstrate that KH2U is a suitable host for selection based on complementation of uracil auxotrophy by addition of an extrachromosomal *pyrE* gene.

Materials and Methods

Strains, media, and growth conditions

Sulfolobus acidocaldarius DSM 639 was grown aerobically at 77°C in Brock's basal salts medium [8] that was adjusted to pH 3.3 with sulfuric acid and supplemented with 0.4% (w/v) of xylose, 0.1% tryptone, and 0.005% yeast extract. For the growth of uracil auxotrophs derived from *S. acidocaldarius* DSM 639, 20 μg/ml of uracil (Sigma) and 50 μg/ml of 5-fluoroorotic acid (Sigma) were added to the medium. To grow *S. acidocaldarius* on solid media, plates were solidified by addition of 0.8% Gelrite (Sigma) and 10 mM $CaCl_2$. Plating technique was performed as previously described [17]. A *mcrBC*-deficient *E. coli* XL Blue MR strain was grown on Luria-Bertani (LB) medium at 37°C. For selection of *E. coli-Sulfolobus* shuttle vector, pC, an ampicillin was supplemented at a concentration of 100 μg/ml. The growth of cells was monitored by measuring the optical density (OD) at 600 nm.

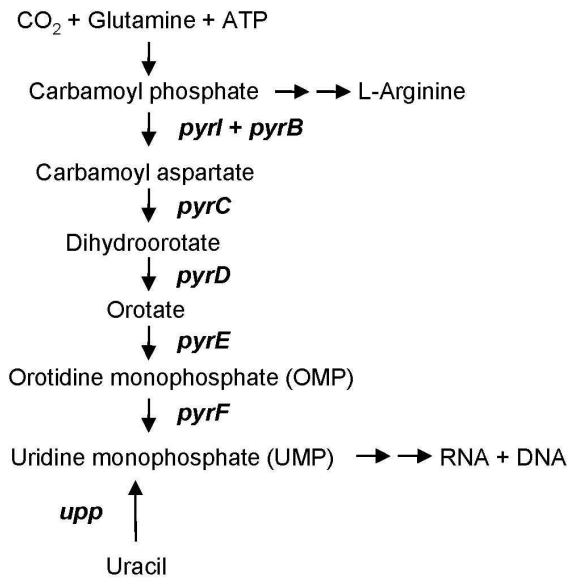

Fig. 1. General mechanism of pyrimidine biosynthesis and genes related to the process in *S. acidocaldarius*. *S. acidocaldarius* synthesizes pyrimidine nucleotides via the intermediates carbamoyl phosphate plus aspartate, carbamoyl aspartate, dihydroorotate, orotate, OMP, and UMP: *pyrI*, aspartate carbomyl transferase; *pyrB*, aspartate carbomyl transferase; *pyrC*, dihydroorotase; *pyrD*, dihydroorotate oxidase; *pyrE*, orotate phosphoribosyltransferase; *pyrF*, OMP decarboxylase; *upp*, uracil phosphoribosyltransferase.

Effect of 5-FOA on growth of *S. acidocaldarius* DSM 639

To select mutants of *S. acidocaldarius* with resistant to 5-FOA, the minimum inhibitory concentration (MIC) of 5-FOA was determined. Cells with an OD at 600 nm of 0.5 were transferred to the various media containing 0 to 100 μg/ml of 5-FOA. During the growth of cells, aliquots from each media were taken at time intervals and monitored by measuring the absorbance at 600 nm.

Survival rate of *S. acidocaldarius* DSM 639 against UV

For the analysis of survival rate by ultraviolet (UV) light, cells with an OD of 0.3 were collected and suspended in one tenth volume of basal salt medium. They were then poured on petri dish and placed under 15 W germicidal lamp (Sankyo Denki, Japan) at a distance of 50 cm. The cells were irradiated for various periods of time. Following irradiation, cells were diluted by serial dilution and spread onto solid medium. The plates were incubated at 75°C for 7 days, and then the number of colonies on plates was counted to determine the survival rates. The survival rates were calculated as follows: the number of isolates with uracil auxotrophy/the number of isolates survived against UV×100.

Isolation of uracil-auxotrophic mutants

In order to obtain spontaneous 5-FOA resistant mutants, cells with an OD of 0.3 were harvested and resuspended in one tenth volume of basal salt medium. After irradiation by UV light for 40 sec, cells were centrifuged and resuspended

in 10 ml of the medium containing uracil (50 μg/ml), and then incubated at 75°C for 2 days. One hundred microliters of irradiated cells were spread onto the plates supplemented with uracil (50 μg/ml) and 5-FOA (50 μg/ml), and then incubated for 10 days. 5-FOA resistant colonies were selected and their uracil auxotrophy was identified by the subculture in liquid medium in the presence or absence of uracil.

Isolation of total DNAs from *S. acidocaldarius*

Cells from 10 ml cultures were centrifuged and resuspended in 250 μl of TEN buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 100 mM NaCl). To lyse the cells, 250 μl of TENST buffer (TEN buffer supplemented with 1.6% sodium-lauroylsarcosyl and 0.12% Triton X-100) was added to the cell suspension and the mixture was incubated for 30 min at room temperature. This solution was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1). Finally sodium acetate (3 M, pH 4.8) was added to the aqueous phase to a concentration of 0.3 M, followed by DNA precipitation with two volumes of ice-cold ethanol. The DNA was washed in 70% ethanol, and dried. The DNA was then dissolved in TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA) containing 10 μg/ml of RNase A.

Sequence analysis of *pyr* gene cluster from uracil-auxotroph mutants

Seven primer sets derived from the sequences of the entire cluster related to pyrimidine biosynthesis in *S. acidocaldarius* DSM 639 were synthesized. The locations amplified by these primers were presented in Fig. 2 and theirs sequences were listed in Table 1. Total 5,969 bp DNA region from chromosomal DNA of mutants was amplified by these primers. The PCR products were ligated into pGEM-T vector (Promega) and their sequences were confirmed by sequencing from Genotech (Daejeon, Korea).

Methylation of *E. coli* plasmid DNA

For transformation of foreign DNA into *S. acidocaldarius*, a *Sulfolobus-E. coli* shuttle vector pC should be methylated at the N4-position of the inner cytosine residue of GGCC recognition sequence to circumvent restriction by the *Sua*I restriction-modification system of *S. acidocaldarius*. The *in vivo* methylation of a shuttle vector pC was carried out by transformation into *E. coli* ER1821 containing the additional plasmid pM.EsaBC4I. Complete methylation was confirmed by the absence of any cutting after treatment with *Had*III restriction enzyme (8 U) for 6 hr.

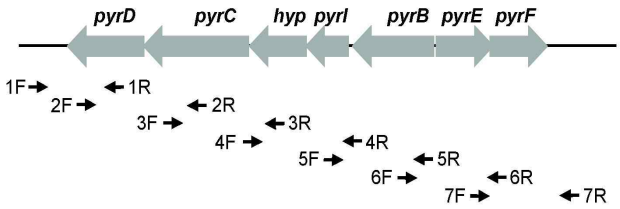

Fig. 2. Relative position and orientation of primers used in sequencing of *pyr* cluster of uracil auxotroph mutants.

Table 1. Sequences of primers used in the study

Primers	Sequences (5′→3′)	Product size (bp)
1F	TGTTGCAACCACAAGTTTACTAAG	1,000
1R	TGTGGAGGAAATAGTTAAGAATGTAAA	
2F	TCATGAATAACTCTTACAGCTAATGC	1,181
2R	TGGGCTTTACCACGGATATAA	
3F	GCTATCCCTGGTGGACAAAG	1,088
3R	TAGGGAAAAGGCTTGGAATG	
4F	CCCATCTTAAATATACGAAAGAGTCA	1,100
4R	GATTGACAGAAAAGTGGACAAGA	
5F	GCAATTCTAAATCCTTCATGTCC	1,054
5R	TTTAAGGGCGTTATTGAAACA	
6F	CGCACCATCGTATTTATGTCTC	1,099
6R	AACCAAGACCTCCTTCTAGCAAT	
7F	GCAGTGGAGGAGGTAAGGAA	994
7R	CCTGACAGGGTATACAAATTAACCA	
PyrE-F	TTTCGTTTTAACATCAGGTAAGG	396
PyrE-R	TGTGAAGCCCCTTCTTGTCT	

Transformation of plasmid into *S. acidocaldarius* and *E. coli*

For preparation of competent cells of *S. acidocaldarius*, cells with an OD of 0.1 to 0.3 on medium containing 0.2% xylose were harvested, washed stepwise in 50 ml, 25 ml and 1 ml of 20 mM sucrose and recovered by centrifugation. The cells were finally resuspended in 200 µl of 20 mM sucrose to a density of 2×10^8 ml^{-1} and 50 µl of the cell suspension was used for electroporation. This cell suspension was added to 1-2 µl (100-300 ng) of the methylated DNA and transferred to an electroporation cuvette with 1 mm gap width (Bio-Rad) on ice. One pulse of electroporation was applied at 1.5 kV, 400 Ω, and 25 µF using a Gene Pulser II (Bio-Rad) electroporator. After pulsing, 50 µl of recovery solution (1% sucrose, 10 mM $MgSO_4$, 0.18% β-alanine and 0.02% DL-malic acid) was immediately added to cuvette and total 100 µl of cell suspension was recovered and incubated for 30 min at 75°C. The cells were spread on solid plates containing 0.2% dextrin and 0.1% tryptone and incubated for 5 days at 75°C. For *E. coli*, transformation was performed by method of rubidium chloride-heat shock and DNA manipulations were carried out as described by Sambrook *et al.* [21]

Results and Discussion

Sensitivity of *S. acidocaldarius* to 5-FOA

To isolate uracil auxotrophic *pyrEF* mutants, MIC values of 5-FOA were determined. 5-FOA metabolites, 5-FOMP (5-fluoroorotidine monophosphate) and 5-FUMP (5-fluorouridine monophosphate), formed by enzymes encoded by *pyrE* and *pyrF* have been reported as inhibitors of cell growth. Therefore, *pyrE* or *pyrF* mutant could be grown on medium containing 5-FOA. The sensitivity of growth inhibition of *S. acidocaldarius* DSM 639 was observed on various concentrations of 5-FOA (Fig. 3A). The growth was completely inhibited at a concentration of 25 µg/ml, and the concentration for selection of spontaneous mutation was used about twice the MIC. By comparison, 0.25 mg/ml 5-FOA was required to inhibit growth of *Halobacterium salinarum* [19].

Isolation of uracil-auxotrophic mutants

UV dosage and exposure time for the spontaneous mutation of *S. acidocaldarius* was determined and UV exposure time suitable for the random mutation was reported that survival rate is about 1% [6]. To determine the survival rate with UV exposure time, viable cells were counted after subjection of UV irradiation for appropriate periods of time (0, 10, 20, 30, 40, 60, 120, 180 s) (Fig. 3B). The survival rates were decreased with an increase of the exposure time and 90, 70, 6, 0.69, 0.64, 0.3 and 0.2% of survival rate was shown for UV exposure time, respectively. At exposure time of 38 s, cell viability was about 1% and this time was used for the mutation of *S. acidocaldarius*.

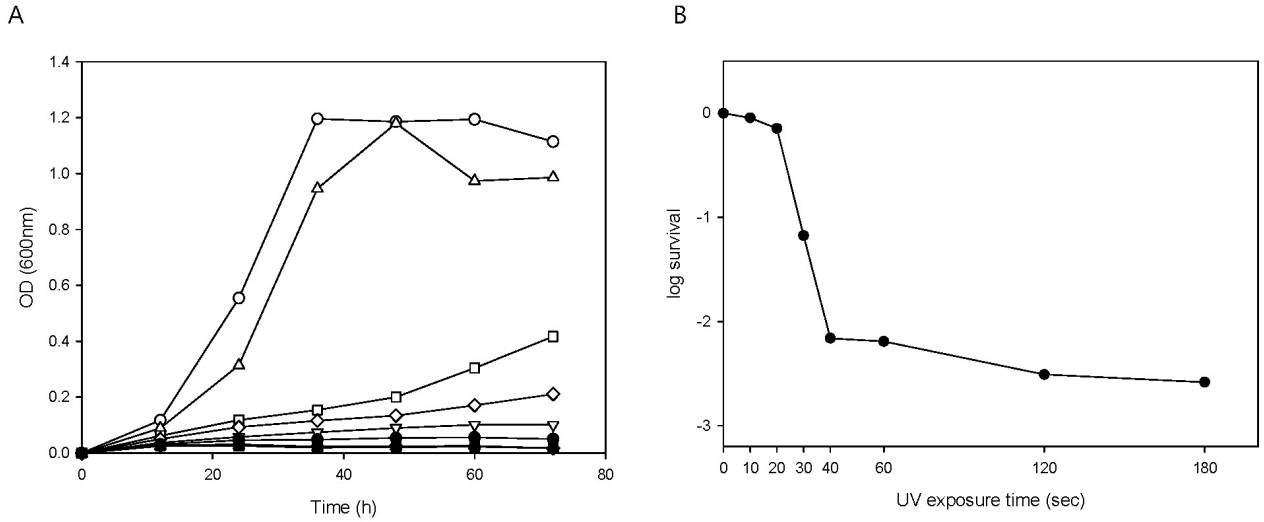

Fig. 3. Determination of MIC of 5-FOA, and UV exposure time for spontaneous mutation of *S. acidocaldarius*. (A) Inhibitory effect of 5-FOA on the growth of *S. acidocaldarius*. Cells were grown on 0.2% xylose medium supplemented with 0 (○), 0.5 (△), 1.0 (□), 2.0 (◇), 2.5 (▽), 5 (●), 25 (▲), 50 (■), and 100 (◆) µg/ml of 5-FOA. (B) Survival rate of *S. acidocaldarius* against UV irradiation. The number of the colonies on 0.2% xylose plate was determined as an interval of exposure time (0, 10, 20, 30, 40, 60, 120, 180 s).

After UV irradiation, 120 colonies of 4.2×10^9 cells were counted on the plate containing 5-FOA and uracil. The 5-FOA resistant colonies were identified by incubation on the medium with or without uracil. Among 120 isolates, 14 isolates harbored uracil auxotrophy and the mutation rate was about 10^{-6}.

Identification of uracil auxotrophic *pyrEF* deficient mutants

To identify whether uracil auxotrophic mutants are caused by inactivation of *pyrEF* gene in pyrimidine biosynthesis gene cluster, the genetic characterization of 2 isolates, KH1U and KH2U, among 14 isolates was examined. The DNA regions containing entire *pyr* cluster from their chromosomal DNA were amplified by PCR with 7 primer sets and mutations were identified by sequencing. As a result, a double point mutation, AAA to CAA (K10Q) at position 28th and GAT to GGT (D112G) at position 335th within *pyrE* gene, and a silent mutation GGA to GGG at position 356th within *pyrF* gene were observed in KH1U mutant (Fig. 4). In case of KH2U mutant, frame shift mutation was occurred by G insertion at position 204th of *pyrE* gene (Fig. 4). Mutations at these sites in both mutants decreased the activity of orotate phosphoribosyltransferase encoded by *pyrE* gene and blocked the degradation of 5-FOA into toxic 5-FOMP and 5-FUMP that kill the cells. Therefore, this result suggested that two mutants, KH1U and KH2U, were spontaneous mutants with uracil auxotrophy. For further *Sulfolobus* genetic studies, KH2U was selected as a host strain because *pyrE* gene was completely inactivated by frame shift mutation of the G insertion.

In order to check the uracil auxotrophy by inactivation of *pyrE* gene, the growth of KH2U on medium containing 0.2% xylose was compared to that of the wild-type cells (Fig. 5). In contrast to the wild-type cells that grow independently with the addition of uracil, KH2U showed a strict uracil

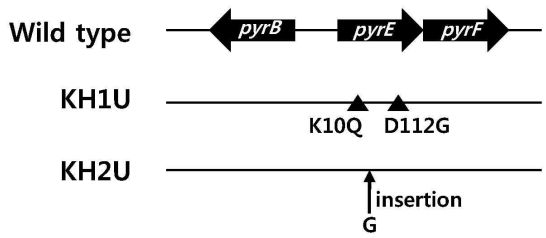

Fig. 4. Mutation sites within *pyrE* gene in KH1U and KH2U mutants.

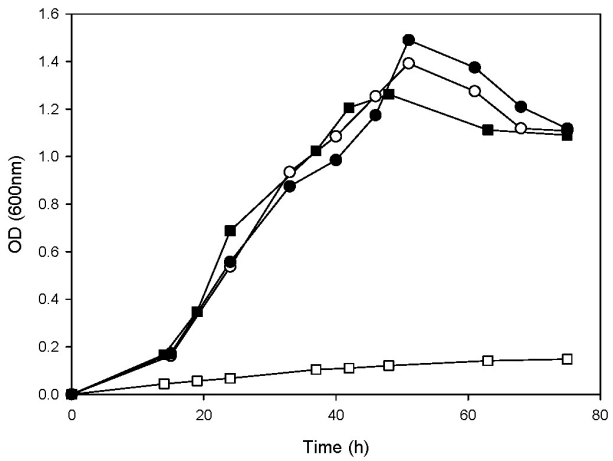

Fig. 5. Growth of *S. acidocaldarius* DSM 639 wild-type and KH2U mutant on the 0.2% xylose medium in the presence or absence of uracil (10 μg/ml). wild-type without uracil (○), wild-type with uracil (●), KH2U without uracil (□), KH2U with uracil (■).

auxotrophy. The growth of the wild-type cells was not affected at all by the presence of uracil while KH2U could be grown when uracil was added in the medium. These results suggested that KH2U was a suitable host strain for genetic studies of *Sulfolobus acidocaldarius*.

Complementation of an uracil auxotrophic mutant KH2U

To identify whether uracil auxotrophy could be complemented by plasmid bearing *pyrEF* genes, KH2U was transformed by *Sulfolobus-E. coli* shuttle vector, pC. The pC is a shuttle vector which carries the replication origin from the pRN1 plasmid in *S. islandicus* REN1H1, and *E. coli* origin and selectable marker from pBluescript [4]. The vector contains the *pyrEF* genes for uracil selection in the *pyrEF* deletion mutant. KH2U was transformed with 100 ng of pC vector by the electroporation as described in Materials and Methods. After incubation for 5 days, Pyr^+ transformants were identified on solid plate lacking uracil (Fig. 6A). Five isolates among all 60 Pyr^+ isolates were randomly selected and the presence of pC vector was examined by colony PCR using specific primer PyrE-F and PyrE-R (Table 1) to identify the *pyrE* gene of pC vector. While five colonies from untransformed KH2U (negative control) were not amplified, all transformed colonies from KH2U were amplified and showed a single band of 400 bp which proves the existence of *pyrE* gene (Fig. 6B).

To confirm whether the pC vector was transformed into

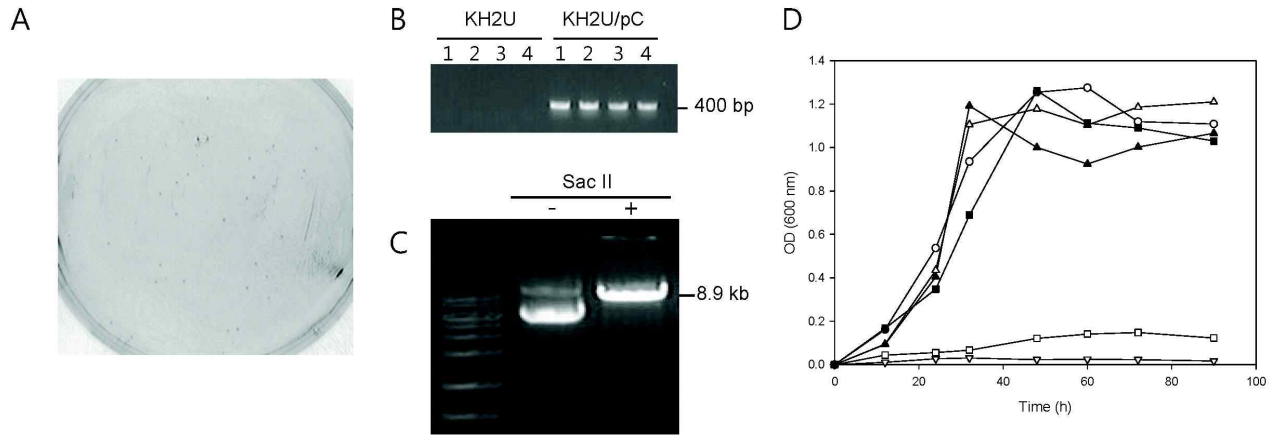

Fig. 6. Complementation of KH2U by transformation of *Sulfolobus-E. coli* shuttle vector pC. (A) Pyr^+ transformants from KH2U on solid medium without uracil. The colonies from KH2U bearing pC vector were observed on medium containing 0.2% dextrin and 0.1% tryptone after electroporation with pC. (B) Colony PCR analysis of untransformed and transformed KH2U with pC. The amplified 400 bp DNA fragment was specific for internal sequence of *pyrE* gene. (C) Identification of pC after retransformation into *E. coli* XL1 Blue MR strain. The plasmid was treated with 10 U of *Sac*II and electrophoresed in an agarose gel. (D) Growth of KH2U and KH2U bearing pC on the 0.2% xylose medium supplemented with or without uracil (10 μg/ml). wild-type cells without uracil (○), KH2U without uracil (□), KH2U with uracil (■), KH2U/pC without uracil (△). KH2U/pC with uracil (▲), KH2U/pC with 5-FOA (▽).

KH2U, total DNA from complemented KH2U strain was extracted and retransformed into *E. coli* XL Blue MR strain. Retransformed plasmid into *E. coli* was digested with *Sac*I restriction enzyme and the resulting 8.9 kbp linearized pC vector was confirmed (Fig. 6C). To examine the growth of complemented KH2U bearing pC on uracil-deficient medium, the wild-type, untransformed KH2U, and transformed KH2U bearing pC were grown on medium in the presence or absence of uracil (Fig. 6D). KH2U was resistant to 5-FOA, whereas it was not grown in uracil-deficient medium. The complemented KH2U overcome the growth inhibition on uracil-free medium by which *pyrEF* gene from pC converted KH2U strain to 5-FOA sensitivity. Thus, we conclude that KH2U is a uracil auxotrophic mutant which cannot grow in uracil-free medium. In addition the expression of *pyrEF* gene in KH2U was restored the ability to pyrimidine biosynthesis and it could overcome uracil auxotrophy.

Presently, it is unclear that one nucleotide insertion within *pyrE* gene in KH2U strain may affect the open reading frame of *pyrF* just located in the downstream of *pyrE* gene. Therefore, the activity of *pyrF* gene should be further analyzed to utilize the uracil auxotrophy gene as an effective selection marker for genetic knockout experiments later. It can be done by the complementation of plasmid with either *pyrE* or *pyrF* gene. We have successfully established a uracil auxotrophic mutant in this study. For the functional genomic studies of this archaeon, the construction of an homologous expression and gene disruption vector system is underway.

Acknowledgements

We thank Georg Lipps for providing *E. coli* ER1821 cell containing pM.EsaBC4I for *in vivo* DNA methylation of pC, and Dennis Grogan for providing *Sulfolobus-E. coli* shuttle vector pC, respectively. This work was supported by National Research Foundation of Korea Grant funded by the Korean Government (KRF-2008-313-F00141).

References

1. Allers, T. and M. Mevarech. 2005. Archaeal genetics: the third way. *Nat. Rev. Genet.* **6**, 58-73.
2. Aravalli, R. N. and R. A. Garrett. 1997. Shuttle vectors for hyperthermophilic archaea. *Extremophiles* **1**, 183-192.
3. Barry, E. R. and S. D. Bell. 2006. DNA replication in the archaea. *Microbiol. Mol. Biol. Rev.* **70**, 876-887.
4. Berkner, S., D. Grogan, S. V. Albers, and G. Lipps. 2007. Small multicopy, non-integrative shuttle vectors based on the plasmid pRN1 for *Sulfolobus acidocaldarius* and *Sulfolobus solfataricus*, model organisms of the (cren-)archaea. *Nucleic Acids Res.* **35**, e88.
5. Berkner, S. and G. Lipps. 2008. Genetic tools for *Sulfolobus* spp.: vectors and first applications. *Arch. Microbiol.* **190**, 217-230.
6. Berkner, S. and G. Lipps. 2008. Mutation and reversion fre-

quencies of different *Sulfolobus* species and strains. *Extremophiles* **12**, 263-270.
7. Blaby, I. K., G. Phillips, E. Blaby-Haas, K. S. Gulig, B. E. Yacoubi, and V. de Crécy-Lagard. 2010. Towards a systems approach in the genetic analysis of archaea: Accelerating mutant construction and phenotypic analysis in *Haloferax volcanii*. *Archaea* **2010**, 426239.
8. Brock, T. D., K. M. Brock, R. T. Belly, and R. L. Weiss. 1972. *Sulfolobus*: A new genus of sulfur-oxidizing bacteria living at low pH and high temperature. *Arch. Microbiol.* **84**, 54-68.
9. Cannio, R., P. Contursi, M. Rossi, and S. Bartolucci. 1998. An autonomously replicating transforming vector for *Sulfolobus solfataricus*. *J. Bacteriol.* **180**, 3237-3240.
10. Deng, L., H. Zhu, Z. Chen, Y. X. Liang, and Q. She. 2009. Unmarked gene deletion and host–vector system for the hyperthermophilic crenarchaeon *Sulfolobus islandicus*. *Extremophiles* **13**, 735-746.
11. Eichler, J. and M. W. W. Adams. 2005. Posttranslational protein modification in archaea. *Microbiol. Mol. Biol. Rev.* **69**, 393-425.
12. Ellen, A., S. V., Albers, and A. J. Driessen. 2010. Comparative study of the extracellular proteome of *Sulfolobus* species reveals limited secretion. *Extremophiles* **14**, 87-98.
13. Facciotti, M. T., D. J., Reiss, M. Pan, A. Kaur, M. Vuthoori, R. Bonneau, P. Shannon, A. Srivasrava, S. M. Donohoe, L. E. Hood, and N. S. Baliga. 2007. General transcription factor specified global gene regulation in archaea. *Proc. Natl. Acad. Sci.* **104**, 4630-4635.
14. Geiduschek, E. P. and M. Ouhammouch. 2005. MicroReview: Archaeal transcription and ts regulators. *Mol. Microbiol.* **56**, 1397-1407.
15. Jun, S. H., M. J., Reichlen, M. Tajiri, and K. S. Murakami. 2011. Archaeal RNA polymerase and transcription regulation. *Crit. Rev. Biochem. Mol. Biol.* **46**, 27-40.
16. Kurosawa, N. and D. W. Grogan. 2005. Homologous recombination of exogenous DNA with the *Sulfolobus acidocaldarius* genome: Properties and uses. *FEMS Microbiol. Lett.* **253**, 141-149.
17. Lindstrom, E. B. and H. M. Sehlin. 1989. High efficiency of plating of the thermophilic sulfur-dependent archaebacterium *Sulfolobus acidocaldarius*. *Appl. Environ. Microbiol.* **55**, 3020-3021.
18. Marintchev, A. and G. Wagner. 2004. Translation initiation: structures, mechanisms and evolution. *Q. Rev. Biophys.* **37**, 197-284.
19. Peck, R. F., S. DasSarma, and M. P. Krebs. 2000. Homologous gene knockout in the archaeon *Halobacterium salinarum* with ura3 as a counterselectable marker. *Mol. Microbiol.* **35**, 667-676.
20. Pritchett, M. A., J. K., Zhang, and W. W. Metcalf. 2004. Development of a markerless genetic exchange method for *Methanosarcina acetivorans* C2A and its use in construction of new genetic tools for methanogenic archaea. *Appl. Environ. Microbiol.* **70**, 1425-1433.
21. Sambrook, J., E. F., Frisch, and T. Maniatis. 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, N.Y.
22. Sato, T., T. Fukui, H. Atomi, and T. Imanaka. 2005. Improved and versatile transformation system allowing multiple genetic manipulations of the hyperthermophilic archaeon *Thermococcus kodakaraensis*. *Appl. Environ. Microbiol.* **71**, 3889-3899.
23. Worthington, P., V. Hoang, F. Perez-Pomares, and P. Blum. 2003. Targeted disruption of the α-amylase gene in the hyperthermophilic archaeon *Sulfolobus solfataricus*. *J. Bacteriol.* **185**, 482-488.
24. Yurist-Doutsch, S., B. Chaban, D. J. Vandyke, K. F. Jarrell, and J. Eichler. 2008. Sweet to the extreme: protein glycosylation in archaea. *Mol. Microbiol.* **68**, 1079-1084.

초록 : *Sulfolobus acidocaldarius* 균주로부터 피리미딘 영양요구주의 분리 및 특성 연구

최경화 · 차재호*
(부산대학교 미생물학과)

고세균 *Sulfolobus acidocaldarius*의 기능유전체학 연구를 위하여 피리미딘 생합성 유전자군의 *pyrEF* 유전자에 근거한 피리미딘 영양요구주를 구축하였다. 원균주는 정상적인 *pyrEF* 존재하에서 5-fluoroorotic acid를 첨가하면 성장이 불가능하나 피리미딘 영양요구주는 성장이 가능한 원리를 활용하였다. 자외선을 이용하여 얻어진 5-FOA 첨가에 저항성을 갖는 돌연변이주를 얻었으며, 두 돌연변이주 KH1U와 KH2U는 각각 *pyrE* 유전자 부분의 점돌연변이와 삽입돌연변이를 갖는 돌연변이주임을 알 수 있었다. 이 두 돌연변이 균주는 5-FOA의 첨가에 의하여 이 세포를 사멸시킬 수 있는 능력이 사라짐을 확인하였다. 정상적인 *pyrEF* 유전자를 갖는 *Sulfolobus-E. coli* 플라스미드를 이용하여 보완실험을 수행한 결과 KH2U 돌연변이주는 다시 5-FOA에 대한 저항성을 잃어버렸으며, 배지 내에 피리미딘의 첨가가 없어도 생존할 수 있는 능력을 보여주는 원균주와 같은 표현형으로 회귀함을 확인하였다. 이 연구는 차후 고세균 *Sulfolobus acidocaldarius*의 유전자 불활성화를 통한 유전학연구에 효율적인 도구로 사용되기에 유용한 연구로 생각된다.