Identification of lateral gene in the genomic DNA sequence, by using the G+C content at the third position of synonymous codons of orthologous genes as an index

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Abstract

All open reading frames (ORFs) of 6 Archaeobacterial and 17 Eubacterial species showed a strong positive correlation between the G+C content (GC content) of the genomic DNA sequences and the G+C content of the third position of the codons (GC3 content). Among them, 1217 pairs of genes that are orthologous between *Pyrococcus horikoshii* OT3 (*Ph*) and *Pyrococcus abyssi* (*Pa*) were identified. The codons of each pair of orthologous genes were classified into three categories, identical codons coding for the same amino acid (IC), different codon coding for the different amino acids (DC) and synonymous codons coding for the same amino acids (IA). In a comparison of the GC3 content of these three types in all orthologous genes between *Ph* and *Pa*, the GC3 content of IA (GC3 content of synonymous codons) deviated the most from the expected value for the GC content of the genome sequences used in this analysis. Therefore the GC3 content of synonymous codon was suggested to be an index that would be able to distinguish a lateral gene from the orthologous genes of the genomic DNA sequence.

By analysis of the GC3 content of synonymous codons, two remarkable regions were found in the genome sequence of *Ph*. In one region between 300 Kbp and 420 Kbp, the genes have a higher GC3 content in the synonymous codon, and in the other between 1,320 Kbp and 1,400 Kbp, the genes have a lower GC3 content in the synonymous codons. In the former region, twelve of sixteen orthologous genes were homologous to genes of Eubacteria or Archaeobacteria. Especially, four orthologous genes showed homology to genes related to synthesis of the cell wall or lipopolysaccharides from *Streptococcus*. These four genes would be suggested to be lateral genes and to represent a lateral region from Eubacterial species. In the latter region, eight out of thirteen orthologous genes were homologous to genes of Archaeobacteria or Eukarya. Six of these eight orthologous genes show homology to the genes related to the function of translation. These genes would be indicated as the stable genes in the progress of evolution, after the divergence between *Pyrococcus horikoshii* OT3 and *Pyrococcus abyssi*.

Key Words: G+C content, synonymous codon, G+C content at the third position of the codon, lateral gene

Area of Interest: Bioinformatics and Bio-Computing

1. Introduction

The number of the genomes whose DNA sequences have been completely determined recently is increasing very rapidly and DNA sequences of more than thirty genomes are now available. By comparison of the genomic DNA sequences between Archaeobacteria and Eubacteria, it was clear that the phylogenetic relationship among them is more complex than expected [1]. Generally, the evolutionary relationship among organisms is explained on the basis of phylogenetic trees derived from the amino acid sequences, coded by genes such as ribosomal RNA genes or other well-conserved genes. But this explanation occasionally leads to a contradiction since different phylogenetic trees would be generated when the different genes are used. To explain this contradiction, a horizontal or lateral gene transfer model was proposed, which stated that in the process of evolution, gene transfer occurred many times among species [2]. Therefore, to better understand the evolutionary relationships among organisms, it is important for such lateral genes to be distinguished from the phylogenetically stable genes of the organism.

One example of lateral gene transfer is the *kfiD* gene, which encodes the K5-specific UDP-glucose dehydrogenase of *Escherichia coli*. The G+C content (GC content) of this gene is much lower than that of the genome's DNA sequence. The pattern of the synonymous codon substitutions between KfiD and Cap3A was different in spite of them having almost equal evolutionary distances. In contrast, the codon usage between KfiD of *E. coli* and that from *Streptococcus* was similar. Based on these results, it was assumed that the *kfiD* gene was transferred from *Streptococcus* into *E. coli* [3]. To identify horizontal gene transfer, however, this method requires a database of homologous genes. And this method can detect only one gene as the horizontally transferred gene. Therefore it is necessary that lateral genes in the genomic DNA sequence are systematically identified by means of an index, which will enable the distinction of a lateral gene from the stable genes.

Bellgard M. I. and Gojobori T. showed that the deviation of the G+C content at the third position (GC3 content) of IA (GC3 content of synonymous codons) of orthologous genes between *Mycoplasma genitalium* and *Mycoplasma pneumoniae* was the largest among the GC3 contents of IC, DC, and IA [4]. They suggested that this bias of the GC3 content of synonymous codons would represent a direct reflection of the evolutionary pressure working on the genome's DNA sequence.

In this paper, we report that the lateral genes among the orthologous genes in *Pyrococcus horikoshii* OT3 were identified, by using the bias of the GC3 content of synonymous codons as the index to distinguish a lateral gene from the stable genes. Two *Pyrococcus* genomic DNA sequences, those of *Pyrococcus horikoshii* OT3 and *Pyrococcus abyssi*, were chosen since their size is small and they are closely related species.

2. Material and Methods

The twenty-three genomic DNA sequences of Archaeobacteria and Eubacteria used in this analysis were downloaded from the NCBI web site (Table1) (<ftp://ncbi.nlm.nih.gov/genbank/genomes/bacteria/>).

Table1. Complete genomic DNA sequences used in this analysis

No.	Organism	Domain*	Size (Mbp)	Abbreviation	References
1	<i>Aeropyrum pernix</i>	A	1.67	Aero	[5]
2	<i>Archaeoglobus fulgidus</i>	A	2.18	Aful	[6]
3	<i>Methanobacterium thermoautotrophicum</i>	A	1.75	Mthe	[7]
4	<i>Methanococcus jannaschii</i>	A	1.66	Mjan	[8]
5	<i>Pyrococcus horikoshii</i> OT3	A	1.80	Pyro	[9]
6	<i>Pyrococcus abyssi</i>	A	1.8	Pabyssi	[10]
7	<i>Aquifex aeolicus</i>	B	1.50	Aquae	[11]
8	<i>Bacillus subtilis</i>	B	4.20	Bsub	[12]
9	<i>Borrelia burgdorferi</i>	B	1.44	Bbur	[13]
10	<i>Chlamydia pneumoniae</i>	B	1.23	Cpnue	[14]
11	<i>Chlamydia trachomatis</i>	B	1.05	Ctra	[15]
12	<i>Deinococcus radiodurans</i>	B	3.28	Dra	[16]
13	<i>Escherichia coli</i> K-12	B	4.60	Ecoli	[17]
14	<i>Haemophilus influenzae</i> Rd	B	1.83	Hinf	[18]
15	<i>Helicobacter pylori</i>	B	1.66	Hpyl	[19]
16	<i>Helicobacter pylori</i>	B	1.64	Hpyl99	[20]
17	<i>Mycoplasma genitalium</i>	B	0.58	Mgen	[21]
18	<i>Mycoplasma pneumoniae</i>	B	0.81	Mpneu	[22]
19	<i>Rickettsia prowazekii</i>	B	1.10	Rpxx	[23]
20	<i>Synechocystis</i> sp.PCC 6803	B	3.57	Synecho	[24]
21	<i>Thermotoga maritima</i>	B	1.80	Tmar	[25]
22	<i>Treponema pallidum</i>	B	1.14	Tpal	[26]
23	<i>Mycobacterium tuberculosis</i> H37Rv	B	4.40	Mtub	[27]

* Characters A and B indicate Archaeobacteria and Eubacteria, respectively.

Both the G+C content of the genomic DNA sequences and the G+C content at each codon position for all ORFs of each species were calculated. All ORFs of *Pyrococcus horikoshii* OT3 (*Ph*) and *Pyrococcus abyssi* (*Pa*) were translated into amino acid sequences. A homology search was done in the non-redundant database (<ftp://ftp.ncbi.nih.gov/blast/db/>) with each translated ORF of *Ph*, using FASTA program [28]. Furthermore, a homology search was done for all the ORFs of *Pa* with each ORF of *Ph* and vice-versa. Pairs of orthologous genes, including one gene from *Ph* and

one gene from *Pa*, were chosen. The pairwise alignment of each pair of orthologous genes was done by using CLUSTAL W [29] and then was applied to calculate the GC3 content in the orthologous genes of the two *Pyrococcus* species. In addition, all ORFs were classified into three domains (Archaeobacteria, Eubacteria and Eukarya), to which the homologous genes belong to and/or into eight categories by function, which the homologous genes have [30].

3. Results and discussion

3.1 The correlation between the G+C content of the genomic DNA sequences and the G+C content at the third codon position (GC3 content) for all ORFs of the genomic DNA sequences

As shown in Fig.1, the G+C content of twenty-three genomic DNA sequences show the range to be between approximately 25 and 70 percent.

Figure 1. Plot of the G+C content between the complete genome sequence (GC content) and the third codon position of all the ORFs (GC3 content). Abbreviations are as listed in Table 1. A regression line is fitted to the data.

There was a strong positive correlation, however, reported between the GC content of the genome sequence and the GC content at each codon position in all ORFs of each genome DNA sequence (*GC3-plot* named in ref [4]) [31]. Nevertheless, the GC3 content in all ORFs demonstrated the best correlation as shown in Fig. 2, which was similar to the result described in ref [32]. Consequently, the use of the GC3 content as a gene index of the gene is suggested to lead to better analytic results, since the genes are thus better patterned and standardized.

Figure 2. Plots of the average GC contents at each codon position for all genes against the G+C contents of the genomic DNA sequences used in this analysis. The regression lines were fitted to the data. Open squares (□), open triangles (△) and filled circles (●) indicate the average G+C content of the first, second and third codon positions, respectively. Abbreviations are as listed in Table1.

3.2 Definition of the orthologous genes

Orthologous genes are those having the same function but existing in the different species. Therefore, it is reasonable to suggest that the amino acids sequences coded by orthologous genes would show high homology and nearly similar lengths. Thus, expectation value obtained from the FASTA homology search (E value), which indicates the probability of the error in the FASTA homology search results, and the ratio of the overlap length between them. To compare the pairwise alignment, pairs comprising of one gene from *Ph* and one gene from *Pa* were chosen. The number of orthologous gene pairs was maximal when the E value was 10^{-20} , and the ratio of the overlap length between a pair of the orthologous genes was 0.7. Each pairwise alignment was performed by using the CLUSTAL W program, and the pattern of the homology between them was confirmed. In this way, 1217 pairs of the orthologous genes were identified. The identified orthologous genes were utilized for further analysis.

3.3 The correlation between the G+C content of the genomic DNA sequences and the G+C content at the third position of the three types of codons found in orthologous genes

Codons compared between orthologous genes were classified into three categories, IA, DA, and IC. IC indicates the identical codons coding the same amino acids; DC indicates the different codons coding the different amino acids; and IA indicates the synonymous codons coding the same amino acids (synonymous codons).

Figure 3. Illustration of the quantities of the differences of GC content used in this analysis. The circles represent the GC3 content for species 1 (*Sp1*) and species 2 (*Sp2*), as labeled. D0 represents the difference of the GC content between *Sp1* and *Sp2*. D1 and D2 indicate the difference of GC content in the overall GC content between *Sp1* and *Sp2* from the *GC3-plot*, respectively. D12 indicates the difference of GC3 content between *Sp1* and *Sp2*.

As shown in Fig. 3, D0 indicates the differences of GC content between species 1 and species 2 and D1, D2 indicate the bias of the GC3 content between species 1 and the value calculated using the regression line at the GC content of species 1 and that of species 2 and the value calculated using the regression line at the GC content of species 2. And D12 showed the difference of GC3 content between species 1 and species 2.

Figure 4. Separate plots of the GC3 content of IA, DC and IC separately, for *Ph/Pa* comparison in GC3-plot. *Ph* is species 1 and *Pa* is species 2. (a) IA category. (b) DA category -only D0 and D12 are shown. (c) IC category – D12 is not shown since D12 is zero by definition.

Fig. 4 shows the correlation between the GC3 content of IC, DC, and IA in the orthologous genes among *Ph* and *Pa* and the GC content of the genomic DNA sequences. The GC3 content of IC is not taken into consideration since the GC3 content does not vary between the two species. The

deviation of the GC3 content of DC was similar to the expectation value of the GC content of the genomic DNA sequences. The deviation of the GC3 content of IA was the largest. Therefore, the GC3 content of the synonymous codon in the orthologous genes was suggested to distinguish lateral gene from the stable genes of the genomic DNA sequence.

3.4 Distribution of the G+C content at the third position of synonymous codons in the orthologous genes of *Ph*

Figure 5 plots the distribution of the GC3 content of synonymous codons in the orthologous genes against the position of the genomic DNA sequence of *Ph*. The average of the GC3 content of synonymous codon in the orthologous genes was 0.365. The genes having synonymous codons with the higher GC3 content were located particularly in the region from approximately 300 Kbp to 420 Kbp (high GC3 content region). And the genes having synonymous codons with the lower GC3 content were located in the region from approximately 1,320 Kbp to 1,400 Kbp (low GC3 content region).

Figure 5. Plot of the GC3 content of synonymous codon of orthologous genes against the genomic DNA sequence of *Ph*. A and B indicate the high GC3 and low GC3 content region, respectively. The overall average of the GC3 content of synonymous codons is 0.365.

As shown in Fig. 6 (a), the distribution of GC3 content of orthologous genes in *Ph* was regarded as a Gaussian distribution having an average and standard deviation of 0.365 and 0.0709, respectively. It was reported that 780 genes in the DNA sequence of *Escherichia coli*, which corresponded to approximately thirty percent of the whole genome sequence, were analyzed by Fractional Correspondence Analysis and the dynamic clustering method and the genes were classified into three classes [33][34]. The 111 genes constituting the horizontally inherited transfer genes comprised the class with the smallest codon bias among the three classes [34]. Therefore, the score of 0.142 with respect to the total genes analyzed, suggests these genes to be transferred in this DNA sequence. When this value is applied to the distribution of GC3 content of the orthologous genes, the coefficient of the standard deviation is approximately 1.5. Thus, the values of the GC3 content were 0.471 and 0.233. For the distribution of the GC3 content of putative lateral genes, it might be likely that the curve would increase and decrease near both edges of the distribution. In addition, patterns of the GC3 content of the synonymous codons in both regions as shown in Figure 6 (b), were different from that of all orthologous genes in the genomic DNA sequence. Therefore, by exploring such values that the distribution curve would increase and decrease near both edges of the distribution curve for GC3 content of orthologous genes, we chose the two values, both 0.475 and 0.245 to distinguish the genes of high GC3 content and low GC3 content, respectively, from the orthologous genes. These values were then applied as thresholds for distinguishing lateral genes from the stable genes of *Ph*.

(a) Histogram of GC3 content of synonymous codon of all orthologous genes

(b) comparison of distribution of GC3 content of synonymous codon

Figure 6. Distribution of the GC3 content of synonymous codons of all orthologous genes in *Ph*.
 (a) The histogram of GC3 content of synonymous codons of all orthologous genes.
 (b) The distribution of GC3 content of synonymous codons of all orthologous genes, the orthologous genes in the region from 300 to 402 Kbp, and those in the region from 1,320 to 1,340 Kbp, as represented by a gray dashed line, a black bold line and a gray bold line, respectively.

In the genomic DNA sequence of *Ph*, there are 78 orthologous genes, whose GC3 content of the synonymous codons is more than or equal to 0.475. Sixteen genes were located in the high GC3 content region in Figure 5, region A. In 48 of the orthologous genes, however, the GC3 content of synonymous codons is less than or equal to 0.245. Thirteen genes were located in the low GC3 content region in Figure 5, region B.

3.5 Characteristics of the high and low GC3 content regions

By analysis of one hundred and twenty-six orthologous genes, forty orthologous genes were homologous to genes from Eubacteria and Archaeobacteria, twenty-four genes from Eukarya and Archaeobacteria and fifty-four from Archaeobacteria. Eight orthologous genes had homologous genes from all three domains, Archaeobacteria, Eubacteria and Eukarya as shown in Fig. 7.

Figure 7. Distribution of the three domains of genes homologous to the orthologous genes in *Ph*. Characters E, A and B indicate Eukarya, Archaeobacteria and Eubacteria, respectively.

In the high GC3 content region shown in Figure 5, region A, 63 genes were orthologous genes, 49 % and 8 % of which showed homology to the genes from Eubacteria and Archaeobacteria and to those of Eukarya and Archaeobacteria, respectively. Twelve of 16 orthologous genes, whose GC3 content of synonymous codons is greater than or equal to 0.475, were homologous to the genes from Eubacteria and Archaeobacteria. In the low GC3 content region shown in Figure 5, region B, 69 genes were orthologous genes, 41 % and 25 % of which were homologues to the genes from Eukarya and Archaeobacteria and from Eubacteria and Archaeobacteria, respectively. Twelve of thirteen orthologous genes, whose GC3 content of synonymous codons is less than or equal to 0.245, were homologous to the genes from Archaeobacteria and Eukarya.

Four orthologous genes in the high GC3 content region, region A as shown in Table 2 (a), were classified into the category of cell structure, because they were homologous to genes related to cell wall or lipopolysaccharides. They also showed high homology to genes related to the cell envelope or biosynthesis of surface polysaccharides and lipopolysaccharides from *Streptococcus*.

On the other hand, the organization of the operon related to this function is known to differ among *Streptococcus thermophilus* species [35]. The 13.6 Kbp *epsL-IS981SC* region of *Streptococcus thermophilus* CNRZ386 is closely related to the functionally similar sequence of *Lactococcus lactis* NIZB40. The IS elements (*ISS1* and *IS981*) shared more than 98 % identity to the homologous *ISS1* and *IS981* from *L. lactis*. In addition, probes isolated from this region hybridized with various DNA fragments of *L. lactis* and the GC content in this region showed variability. This suggests that the entire *epsL-IS981SC* region was transferred from *L. lactis* [35]. The four orthologous genes had a bias of GC3 content of synonymous codons and were homologous to the genes from Eubacteria and Archaeobacteria in Fig. 8 (b), region A. We found that they are likely to be lateral genes.

Table 2. Number of the GC3 content of synonymous codons of orthologous genes in *Ph*

(a) GC3 content of synonymous codons of the orthologous genes is 0.475 or greater.

category of function	number of the orthologous genes	number of the orthologous genes in the 300-420 Kbp (region A)
transcription	3	0
translation	5	0
replication	5	1
cell process	5	1
cell structure	8	4
metabolism	7	2
others	0	0
hypothetical protein	45	8

(b) GC3 content of synonymous codons of the orthologous genes is 0.245 or less.

category of function	number of the orthologous genes	number of the orthologous genes in the 1,320-1,400 Kbp (region B)
transcription	2	0
translation	11	6
replication	0	0
cell process	3	1
cell structure	1	0
metabolism	5	1
others	1	0
hypothetical protein	25	5

(a) orthologous genes in the high GC3 region

gene	domain	description
PH_0330		hypothetical protein
PH_0331		hypothetical protein
PH_0332		Nucleotide Metabolism; Purine metabolism
PH_0334		hypothetical protein
PH_0335		hypothetical protein
PH_0337		hypothetical protein
PH_0340		pepX related from <i>Caldicellulosiruptor saccharolyticus</i>
PH_0341		hypothetical protein
PH_0342		hypothetical protein
PH_0344		hypothetical protein
PH_0343		hypothetical protein
PH_0345		hypothetical protein
PH_0346		hypothetical protein
PH_0347		hypothetical protein
PH_0349		hypothetical protein
PH_0352		hypothetical protein
PH_0353		GTP-binding ERA related
PH_0355		hypothetical protein
PH_0356		hypothetical protein
PH_0357		glucan-1-transferase
PH_0359		hypothetical protein
PH_0360		hypothetical protein
PH_0362		hypothetical protein
PH_0363		hypothetical protein
PH_0364		hypothetical protein
PH_0365		geranylgeranyl hydrogenase related
PH_0366		hypothetical protein
PH_0367		hypothetical protein
PH_0368		glutamine synthetase (glnA)
PH_0372		ribonucleotide reductase (nrd)
PH_0381		hypothetical protein
PH_0382		cobalamin (5'-phosphate) synthase (cobS-1)
PH_0383		hypothetical protein
PH_0384		hypothetical protein
PH_0385		cobalamin biosynthesis protein B
PH_0386		histidinol-phosphate aminotransferase (hisC)
PH_0388		UDP-glucose 4-epimerase (galE-2)
PH_0390		glucose-1-phosphate thymidyltransferase (graD-1)
PH_0391		hypothetical protein
PH_0393		hypothetical protein
PH_0394		hypothetical protein
PH_0397		hypothetical protein
PH_0400		polysaccharide biosynthesis protein, putative
PH_0411		glycosyl transferase
PH_0412		hypothetical protein
PH_0435		dTDP-glucose 4,6-dehydratase (rfbB)
PH_0437		DTDP-4-DEHYDRORHAMNOSE 3,5-EPIMERASE (EC 5.1.3.13)
PH_0438		DTDP-4-DEHYDRORHAMNOSE REDUCTASE
PH_0442		polysaccharide biosynthesis protein, putative
PH_0454		dolichol-P-glucose synthetase
PH_0455		Lps biosynthesis rfbU related protein
PH_0456		SUA5 related protein
PH_0458		hypothetical protein
PH_0459		adenylosuccinate synthetase (purA)
PH_0460		COMPETENCE-DAMAGE PROTEIN
PH_0461		putative, translation initiation factor aIF-2BII translation initiation factor
PH_0463		DNA MISMATCH RECOGNITION PROTEIN MUTS.
PH_0466		O-sialoglycoprotein endopeptidase, putative
PH_0467		hypothetical protein
PH_0468		hypothetical protein
PH_0469		hypothetical protein
PH_0470		hypothetical protein
PH_0471		DNA repair protein RAD25
PH_0472		hypothetical protein
PH_0473		ATP-dependent protease La (lon)
PH_0477		hyaluronan synthetase related
PH_0478		glyceraldehyde-3-phosphate
PH_0479		hypothetical protein
PH_0480		2-haloalkanoic acid dehydrogenase related protein
PH_0481		CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase (pgsA)
PH_0482		hypothetical protein
PH_0483		hypothetical protein
PH_0484		hypothetical protein
PH_0485		hypothetical protein
PH_0487		thermostable carboxypeptidase
PH_0488		hypothetical protein
PH_0491		hypothetical protein
PH_0493		stomatin-like protein
PH_0494		hypothetical protein
PH_0495		hypothetical protein
PH_0496		Na ⁺ /Ca ²⁺ exchanging protein related
PH_0499		hypothetical protein

300-420 Kbp
(region A)

(b) genes in the region A of the high GC3 region

ORF	domain	description
PH_0340		pepX related from <i>Caldicellulosiruptor saccharolyticus</i>
PH_0341		hypothetical protein
PH_0343		hypothetical protein
PH_0344		hypothetical protein
PH_0345		hypothetical protein
PH_0346		hypothetical protein
PH_0347		hypothetical protein
PH_0348		hypothetical protein
PH_0349		hypothetical protein
PH_0350		
PH_0351		
PH_0352		hypothetical protein
PH_0353		GTP-binding protein ERA related
PH_0354		
PH_0355		hypothetical protein
PH_0356		hypothetical protein
PH_0357		UDP-N-acetylglucosamine-1-phosphate transferase
PH_0358		
PH_0359		hypothetical protein
PH_0360		hypothetical protein
PH_0361		
PH_0362		hypothetical protein
PH_0363		hypothetical protein
PH_0364		hypothetical protein
PH_0365		geranylgeranyl hydrogenase related
PH_0366		hypothetical protein
PH_0367		hypothetical protein
PH_0368		glutamine synthetase (glnA)
PH_0369		hypothetical protein
PH_0370		integral membrane protein
PH_0371		
PH_0372		ribonucleotide reductase (nrd)
PH_0373		
PH_0374		galactose 1 phosphate uridyl transferase
PH_0375		beta glucosidase
PH_0376		hypothetical protein
PH_0377		alpha amylase 1
PH_0378		galactokinase
PH_0379		hypothetical protein
PH_0380		hypothetical protein
PH_0381		hypothetical protein
PH_0382		cobalamin (5'-phosphate) synthase (cobS-1)
PH_0383		hypothetical protein
PH_0384		hypothetical protein
PH_0385		cobalamin biosynthesis protein B
PH_0386		histidinol-phosphate aminotransferase (hisC)
PH_0387		
PH_0388		UDP-glucose 4-epimerase (galE-2)
PH_0389		hypothetical protein
PH_0390		glucose-1 phosphate transferase
PH_0391		hypothetical protein
PH_0392		hypothetical protein
PH_0393		hypothetical protein
PH_0394		hypothetical protein
PH_0395		methanol dehydrogenase regulatory protein (moxr) protein
PH_0396		
PH_0397		hypothetical protein
PH_0398		
PH_0399		hypothetical protein
PH_0400		polysaccharide biosynthesis protein, putative
PH_0401		
PH_0402		hypothetical protein
PH_0403		
PH_0404		4 hydroxybenzoate octaprenyltransferase (ubia)
PH_0405		
PH_0406		N-acetylglucosaminyltransferase
PH_0407		teichoic acid biosynthesis protein
PH_0408		hypothetical protein
PH_0409		hypothetical protein
PH_0410		
PH_0411		glycosyl transferase
PH_0412		hypothetical protein
PH_0413		hypothetical protein
PH_0414		
PH_0415		
PH_0416		
PH_0417		
PH_0418		
PH_0419		hypothetical protein
PH_0420		hypothetical protein
PH_0421		
PH_0422		
PH_0423		
PH_0424		
PH_0425		hypothetical protein
PH_0426		
PH_0427		
PH_0428		
PH_0429		hypothetical protein
PH_0430		
PH_0431		
PH_0432		hypothetical protein
PH_0433		
PH_0434		glucose 1 phosphate thymidyltransferase
PH_0435		dTDP-glucose 4,6-dehydratase (rfbB)
PH_0436		
PH_0437		DTDP-4-dehydrorhamnose 3,5-epimerase
PH_0438		DTDP-4-dehydrorhamnose reductase
PH_0439		
PH_0440		
PH_0441		
PH_0442		polysaccharide biosynthesis protein, putative
PH_0443		
PH_0444		
PH_0445		rhmannosyl transferase epsq protein
PH_0446		UDP galactopyranose mutase
PH_0447		lipopolysaccharide N acetylglucosaminyltransferase
PH_0448		
PH_0449		
PH_0450		wbpA B-band liposaccharide gene cluster
PH_0451		glycosyl transferase
PH_0452		hypothetical protein
PH_0453		rhmannosyl transferase
PH_0454		dolichol-P-glucose synthetase
PH_0455		Lps biosynthesis rfbU related protein epsr protein
PH_0456		SUA5 related protein
PH_0457		
PH_0458		hypothetical protein
PH_0459		adenylosuccinate synthetase (purA)
PH_0460		competence-damage protein
PH_0461		putative, translation initiation factor aIF-2BII translation initiation factor
PH_0462		
PH_0463		DNA mismatch recognition protein MUTS.
PH_0464		methyl-accepting chemotaxis protein
PH_0465		
PH_0466		O-sialoglycoprotein endopeptidase, putative
PH_0467		hypothetical protein
PH_0468		hypothetical protein
PH_0469		hypothetical protein
PH_0470		hypothetical protein
PH_0471		DNA repair protein RAD25
PH_0472		hypothetical protein
PH_0473		ATP-dependent protease La (lon)
PH_0474		
PH_0475		
PH_0476		
PH_0477		haluronan synthetase related
PH_0478		glyceraldehyde-3-phosphate
PH_0479		hypothetical protein
PH_0480		2-haloalkanoic acid dehydrogenase

region 1

(continued)

Figure 8. Gene maps of the high and low GC3 content region.

(a) and (b) show the high content region and (c) and (d) show the low content region.

Red blocks indicate the homologues from Archaeobacteria. Blue ones indicate those from Eubacteria and Archaeobacteria. Green ones indicate those from Eukarya and Archaeobacteria. Gray ones indicate those from Eukarya, Eubacteria and Archaeobacteria. Violet ones indicate that from Eukarya and Eubacteria. Yellow squares represent the

genes having the higher GC3 content of synonymous codons, greater than or equal to 0.475, in region A of the high GC3 content region and those having the lower GC3 content, lower than or equal to 0.245, in region B of the low GC3 content region. Gray squares represent the orthologous genes. In the region 1, the orthologous genes were related to cell-wall or lipopolysaccharides and in region B, the orthologous genes were related to translation.

We considered that the region from PH_0388 and PH_0455 in Fig. 8 (b), region 1, would have transferred into *Ph* for the following four reasons. In the high GC3 content region, orthologous genes having the higher GC3 content of synonymous codon were located around PH_0388 and PH_0455. In the region A, four genes of function related to cell wall as shown in Table 2 (a), were located at both end of the region 1. They showed high homology to the genes related to the cell wall or lipopolysaccharides from *Streptococcus*, which were supposed to have been transferred from *L. lactis*. The genes in this region 1 were homologous to the genes of Eubacteria and Archaeobacteria and included 16 genes related to the cell wall or lipopolysaccharides. Moreover, there were many genes without any homologues attributable to the closely related species, *Pyrococcus abyssi*. Taken together, the region from PH_0388 to PH_0455 is suggested to be a lateral region from Eubacteria.

In the low GC3 content region in Fig. 8 (c), region B, six orthologous genes were classified into the category of translation as shown in Table 2 (b). Among these six, five were homologous to genes coding for ribosomal proteins. As shown in Fig. 8 (c), all of the orthologous genes except PH_1529, whose GC3 content of synonymous codons is less than or equal to 0.245, were homologous to genes from Archaeobacteria and Eukarya. It is just conceivable that mutations had been occurring in the nucleotide bases, and as the result of mutation, amino acids were changed or not. In spite of the selection pressure in evolution, changes have been taking effect not on amino acids or proteins but rather on nucleotide bases or the chromosomes. Thus, the smallest deviation from the GC content of the genomic DNA sequence, in comparison with that of other regions, demonstrates that the genes were mostly unaffected in evolution.

Taken together, it is suggested that a region from another species would have transferred into the 300-420 Kbp region of the genomic DNA sequence of *Pyrococcus horikoshii* OT3, since eight putative lateral genes were located in this region. Additionally, fifteen putative lateral genes were identified in the high GC3 content region.

The index value given to the G+C content at the third position of the synonymous codons in the orthologous genes enables us to identify a gene as a lateral gene existing among the genes that would have been affected with respect to on base composition but have not been directly affected with respect to amino acids in evolution. Since such genes could be removed during species relationship analysis, a better phylogenetic tree could be produced that would aid in more properly understanding the phylogenetic relationship. We applied this method to analyze *Pyrococcus horikoshii* OT3 and identified genes and a region from Eubacteria as the respective lateral genes and a lateral region within the genome DNA sequence.

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