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Research Article

Does CRISPR/Cas Help Us to Understand Microbial Ecology in Space?:*Thermococcus* spp. Samples

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ABSTRACT

In this research, CRISPR/Cas regions of 27 whole-genome sequences of hyperthermophilic archaea, *Thermococcoccus* spp. were examined. Spacer sequences obtained from CRISPR/Cas systems were screened and plasmid or phage invaders were detected. Hydrothermal vents have enormous microbial diversity and chemical composition. So, deep-sea vents are very critical localizations to study and observe early earth's environment and evolution. Therefore, databases of spacers derived from the clustered regularly interspaced short palindromic repeat (CRISPR) systems were used to see whether this system can be used to guess ecological interactions between archaea and viruses or plasmids. These hydrothermal vents have been thought to be found in other ocean worlds such as Europa and Enceladus. Therefore, astrobiologists have been quite interested in these moons, and researchers have made thought about whether these systems may be evidence of extremophiles. In conclusion, CRISPR/Cas system has been thought to provide a further step to observe ecological diversity in extraterrestrial systems, especially the ones that are assumed to have hydrothermal vents such as in Europa or Enceladus.

Keywords: CRISPR/Cas; *Thermococcus*; Europa;hydrothermal vents; Enceladus.

INTRODUCTION

CRISPR/Cas system (Clustered regularly interspaced short palindromic repeats–CRISPR associated genes) provides bacterial immunity to protect themselves from phage or plasmid attacks [1]. 90% of archaea have a CRISPR system [2]. CRISPR/Cas system consists of two classes, Class 1 and Class 2, six types (I-VI), and 34 subtypes [1]. Cas genes composition, repeat sequences, and crRNA-effector complex designates this classification. A CRISPR array includes direct repeats and spacers, small DNA pieces from phages, or plasmids that are incorporated into direct repeats [3]. Repeat sequences are conserved short sequences in length 20 to 40 bases. Spacer sequences can be clue sequences to guess interactions of bacteria or archaea with their environments since these small DNA pieces come from either phage or plasmid invaders [4,5]. Therefore, CRISPR locus acts as the memory center of archaea or bacteria [6]. Recently, spacer sequences obtained from CRISPR arrays have been used to study ecological interactions of bacteria [4,1].

Extremophiles are organisms that live in extreme environmental conditions such as high salinity, high temperature, high alkalinity, high radiation, and desiccation, etc [7]. These microorganisms are good candidates to study life in space since they have been thought to endure extreme conditions existing out of the earth. So, hyperthermophiles, which can grow above 80°C, are one of the model organisms to study extraterrestrial life [8,7]. Hydrothermal vents are the model localizations to study early environments and the evolution of the earth [9]. These vents harbor hyperthermophilic archaea [10]. *Thermococcus* sp. is a genus of Euryarchaeote in the family Thermococcaceae [11]. These microorganisms are obligate anaerobic chemoorganotrophs that use elemental sulfur as an electron acceptor.

Hydrothermal vents have been thought to find in other ocean worlds such as Europa and Enceladus [12]. Therefore,

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astrobiologists have been interested in these moons, and researchers have made thought about whether these systems may be evidence of microorganisms [13].

This study investigated whether CRISPR/Cas system can be a good sign to guess environmental interactions of archaeal species if they are found in outer space. Therefore, in this study, *Thermococcus* spp. was selected as model organisms that are mostly isolated from hydrothermal vents. 27 whole genomes were screened for CRISPR/Cas systems and spacers. Spacer sequences were furtherly analyzed to inspect phage or plasmid interactions of archaeal species.

MATERİALS AND METHODS

Complete genome sequences

27 whole-genome sequences were downloaded from NCBI (National Center for Biotechnology Information Genome Bank (last access, August 2021).

CRISPR/Cas system identification

CRISPR/Cas systems, and spacers of *Thermococcus* spp. genomes were predicted by using two common software tools, CRISPRfinder and CRISPR-Cas ++, according to the default settings. Having at least four spacers within an array was selected as a parameter for further analysis and spacers were downloaded from CRISPR-Cas ++ as FASTA format.

Spacer analysis of genomes

CRISPR spacers were used as a query for the CRISPRTarget analysis tool and used to search against a database limited to Refseq-Plasmid and Phage by giving a cut-off score of 20. Redundant spacers were removed by the tool when the input had multiple spacers from several related species. According to the report of the target tool, spacer sequences were also examined manually in NCBI. Spacer scores are presented in Table 1, matches under 20 base pairs were not considered.

RESULTS

Twenty-seven whole genomes of *Thermococcus* spp. were examined. 26 strains have been found to have CRISPR/Cas system except for one species, namely, T. celericrescens. 21 species had type I-B, 12 species had I-A, 7 species had III-A, 6 species had III-B and 1 species had III-D (Table 1). According to spacer analysis, the maximum spacer number was 82 and the lowest was 4. Spacer analysis results revealed plasmid invaders of *Thermococcus* sp. and one virus invader belonging to T. barophilus. T. gammatolerans EJ 3 spacer analysis revealed that Paraoceanicella profunda strain D4M 1 plasmid pD4M 1C is the invader. Paraoceanicella profunda is a novel piezophilic alphaproteobacterium isolated from deep sea water of the Mariana Trench [14]. Spacer sequence of T. barophilus CH 5 matched with Pyrococcus abyssi virus 1, which is an archaeal dsDNA virus infecting Pyrococcus abyssi, a hyperthermophilic Euryarchaeota [15]. Spacer sequences of T. nautili 30-1 matched with three plasmids belonging to *Thermococcus* sp., namely, *Thermococcus* sp. EXT9 plasmid pEXT9a, *Thermococcus* sp. AMT 7 plasmid pAMT 7, and *Thermococcus* sp. IRI 33 plasmid pIRI33. Spacer sequence of T. indicus IOH1 matched with *Thermococcus* sp. IRI48 plasmid pIRI48 (Figure 1). Spacer sequence of T. piezophilus CDGS matched with Thermobacillus composti KWC4 plasmid pTHECO01. Spacer sequence of T. cleftensis CL1 matched with Pyrococcus abyssi strain GE2 plasmid pGE2.

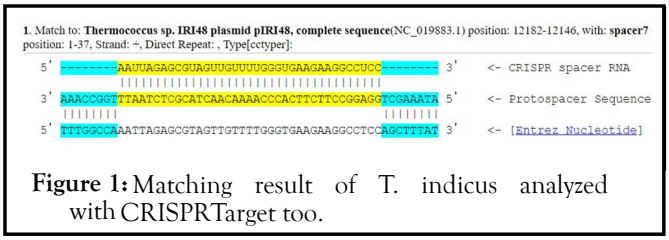

Figure 1: Matching result of T. indicus analyzed with CRISPRTarget too.

Table 1: Accession numbers, CRISPR/Cas systems, types, spacer numbers, and phage/plasmid invaders corresponding to a spacer sequences of Thermococcus spp.

Thermococcus spp.	Strain	CRISPR type	Max Spacer Number	Phage/Plasmid invaders	Spacer Score	Accession Number	
1	T. kodakarensis	KOD1	I-A, I-B	36	-	-	NC_006624.1
2	T. sibiricus	MM 739	I-B	24	-	-	NC_012883.1
3	T. gammatolerans	EJ3	I-A	21	Paraoceanicella profunda strain D4M1 plasmid pD4M1C	37/23	NC_012804.1
4	T. onnurineus	NA1	III-D	43	-	-	NC_011529.1
5	T. barophilus	CH5	I-B, III-B	25	Pyrococcus abyssi virus 1	38/22	NZ_CP013050.1
6	T. barophilus	MP	I-A	25	-	-	NC_014804.1

7	T. zilligii	AN1	III-B, III-A, I-B, I-A	57	-	-	NZ_AJLF00000000.1
8	T. litoralis	DSM 5473	I-A, I-B	68	-	-	NC_022084.1
9	T. peptonophilus	OG-1	I-B	32	-	-	NZ_CP014750.1
10	T. guaymasensis	DSM 11113	I-A, I-B	30	-	-	NZ_CP007140.1
11	T. thioreducens	OGL-20P,	I-A	7	-	-	NZ_CP015105.1
12	T. celer	Vu 13	I-B	14	-	-	NZ_CP014854.1
13	T. paralvinellae	ES1	I-B	33	-	-	NZ_CP006965.1
14	T. nautili	30-1	I-B	54	*Thermococcus* sp. EXT9 plasmid pEXT9a		
***Thermococcus* sp. AMT7 plasmid pAMT7**							
Thermococcus sp. IRI33 plasmid pIRI33	37/31						
36/32							
36/32	NZ_CP007264.1						
15	T. camini	IRI35c	I-B	34	-	-	NZ_LR881183.1
16	T. stetteri	DSM 5262	I-A, I-B, III-A	28	-	-	NZ_JAGGKB000000000.1;
17	T. indicus	IOH1	I-B, III-A, III-B	82	*Thermococcus* sp. IRI48 plasmid pIRI48	37/37	NZ_CP040846.1
18	T. profundus	DT 5432	I-A	30	-	-	NZ_CP015103.1
19	T. radiotolerans	EJ2	I-B, III-A	13	-	-	NZ_CP014862.1
20	T. siculi	RG-20	I-A, I-B, III-B	44	-	-	NZ_CP015106.1
21	T. pacificus	P-4	I-B	4	-	-	NZ_CP015102.1
22	T. barossii	SHCK-94	I-A, I-B	20	-	-	NZ_CP015101.1
23	T. piezophilus	CDGS	III-A, I-B	35	Thermobacillus composti KWC4 plasmid pTHECO01	37/23	NZ_CP015520.1
24	T. chitonophagus	1	I-B, III-A, III-B	29	-	-	NZ_LN999010.1
25	T. celericrescens	DSM 17994	-	-	-	-	NZ_LLYW01000029.1
26	T. cle tensis	CL1	I-B, III-A	38	Pyrococcus abyssi strain GE2 plasmid pGE2	37/25	NC_018015.1
27	T.eurythermalis	A501	I-A, I-B, III-B	71	-	-	NZ_CP008887.1

DISCUSSION

The CRISPR/Cas system provides critical clues on interactions of bacteria or archaea with their invaders. In this study, results indicated that spacer sequences obtained from CRISPR/Cas systems can provide an ecological estimation between species in the same niche. Additionally, the abundance of spacer sequences suggests that these microorganisms expose viral and plasmid attacks in their environment.

In a previous study, viral assemblages in the hydrothermal vents were used to find ecological hosts through CRISPR/Cas system spacer analysis. But, in this study, for the first time, the CRISPR/Cas system of a host species, namely, *Thermococcus* sp. was analyzed to find which species or viruses can be found and invaded archaea in the same ecological environment. Previous studies have suggested the possible existence of microbial life in extraterrestrial environments such as the planet Mars and Jupiter's moon Europa due to the survival of extremophiles under simulated interplanetary conditions [16].

CONCLUSION

Hydrothermal vents are special environments since they contain tremendous chemical composition as well as microbial diversity. Therefore, especially, black smokers have been studied to understand early earth conditions and evolution. These hydrothermal vents have been thought to find in other ocean worlds such as Europa and Enceladus. Therefore, astrobiologists have been quite interested in these moons, and researchers have made thought about whether these systems may be evidence of microorganisms. In conclusion, CRISPR/Cas system has been thought to provide a further step to observe ecological diversity in extraterrestrial systems, especially the ones that are assumed to have hydrothermal vents such as in Europa or Enceladus.

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