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Use of the DiversiLab repetitive sequence-based PCR system for genotyping and identification of Archaea

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Abstract

Repetitive elements are short stretches of DNA that are randomly distributed throughout the chromosomes of prokaryotes. The use of PCR primers to amplify intervening sequences of DNA between specific repetitive elements in Bacteria has become a standard method for rapidly genotyping bacterial strains and providing good resolution between multiple strains within a single species. Rapid, standardized methods for high resolution genotyping of Archaea are not widely available. We evaluated the DiversiLab system from Bacterial Barcodes that utilizes a kit-based repetitive sequence-based (rep-PCR) method that has been optimized for genotyping DNA was extracted from the source organisms using either a standard chemical DNA extraction kit or Whatman FTA® paper. Rep-PCR was performed using an archaeal primer set and, the products were run on an Agilent, Lab-on-a-Chip DNA analyzer. Results were analyzed and compared using DiversiLab web-based software from Bacterial Barcodes. Seventy-nine strains representing 27 genera of *Crenarchaeota* and *Euryarchaeota* were analyzed. All the organisms could be successfully genotyped and the results were reproducible. We could not detect differences in rep-PCR profiles between DNA extracted using the chemical extraction kit and FTA® paper. Thus far, 14 genera and 32 species of methanogens have been analyzed, and all yielded unique genotypes. For halophiles, 11 genera and 28 different species were analyzed, and all yielded unique genotypes. A comparison of 7 different strains of *Halobacterium salinarium* demonstrated that 6 of the 7 strains had a unique genotype. A comparison of 4 strains of *Methanosarcina mazei* indicated that each strain produced a unique genotype. There was little systematic inference that could be made from dendrograms comparing different strains, species, and genera of Archaea based on UPGMA cluster analysis. Based on these results, rep-PCR was a useful tool for the genotyping and strain identification of Archaea.

Keywords: Sequence-based; PCR; Archaea

1. Introduction

Most methods for rapid and systematic identification of prokaryotes have been developed primarily for the Bacteria and not the Archaea. As a result, the techniques tend to be specific for bacteria, for example fatty acid analysis and automated riboprinting. Identification techniques for archaea are more limited to DNA sequence-based phylogenetic analysis of the small subunit rRNA gene or other phylogentically conserved genes. While these are excellent means of classification, in general, they are not capable of strain-level resolution, and can be relatively laborious to perform. Mass spectrometry has been used for rapid identification of archaea with good results; however mass spectrometry measures phenotypic properties of cells, and as such requires a uniform physiological cell state to yield consistent results (Krader and Emerson, 2004). Strain-specific genotyping methods for archaea are not well established. The use of random amplified polymorphic DNA (RAPD) fingerprinting has been applied to determining the population structure among *Thermococcales* from hydrothermal vents (Lepage et al., 2004); however we are aware of few other studies that have applied genotyping techniques to archaea. With

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increasing numbers of archaea being described there is a need for rapid, economical identification methods that provide strain-level discrimination across diverse taxa.

Repetitive sequence-based PCR (rep-PCR), also referred to as repetitive element PCR and repetitive extragenic palindromic-PCR is a genotyping technique that has been used for over 10 years on a variety of bacteria with much success (Versalovic et al., 1994). This method takes advantage of short repetitive oligonucleotide elements that are scattered throughout the genomes of prokaryotes. By using PCR primers specific for these repetitive sequences it is possible to amplify the intervening regions of DNA. When these DNA fragments are separated electrophoretically they form a fingerprint or barcode that is unique for a given strain. Rep-PCR has become a standard method for rapidly genotyping bacterial strains and providing good resolution between multiple strains within a single species (Healy et al., 2005). However, its use has been confined primarily to bacterial taxa that are of importance to human, animal, or plant health. Relatively little work has been done to determine the efficacy of using rep-PCR to identify the broader diversity of prokaryotes that account for the large majority of environmental microbes. Uses on purely environmental isolates include comparison of *Loktanella* spp. isolates from microbial mats in Antarctica (Van Trappen et al., 2004), *Thermomononas* spp. from a bioreactor (Mergaert et al., 2003), and a *Halomonas* sp. from a microbial biofilm (Heyrman et al., 2002). Other examples of using rep-PCR to determine relationships among microbial populations from specific habitats include an analysis of *Bacillus* endospores isolated from granite (Fajardo-Cavazos and Nicholson, 2006), halophilic sulfur-oxidizers (Foti et al., 2006), pulp mill wastewater treatment systems (Baker et al., 2003), and the International Space Station (Castro et al., 2004).

We evaluated a rep-PCR kit from Bacterial Barcodes (a division of Biomeriuex) that has been optimized for genotyping Archaea. The method involves extracting DNA from the source organisms, performing a PCR-based amplification using an archaeal primer set provided in the kit, and then separating the amplicons based on size using an Agilent Lab-on-a-Chip DNA analyzer. Results were analyzed and compared using a web-based analysis system (Bacterial Barcodes, www.bacbarcodes.com). In this study we evaluated the system for its ability to aid in the identification of a diverse set of prokaryotes with a focus on halophilic archaea and methanogens.

2. Materials and methods

2.1. Bacterial strains

All strains analyzed were obtained from ATCC and propagated on recommended media under described conditions, and are shown in Table 1. Media formulations and growth conditions can be found by searching under the organism name or ATCC number on the ATCC website, www.atcc.org.

2.2. Genomic DNA extraction

Cells were harvested in the late log phase of growth from 1.8 to 10 ml of broth, or, in a few cases, as 3–5 colonies from an agar

Table 1
List of strains tested in this study

Genus/species	ATCC no.	Genus/species	ATCC no.
Archaea			
Haloarcula		*Methanothermobacter*	
quadrata	700850	*thermautotrophicus*	700791
marismortui	43049	*marburgensis*	BAA-927
hispanica	33960	*thermophilus*	BAA-1076
vallismortis	29715	*Methanocalculus*	
japonica	49778	*pumilus*	BAA-129
argentinensis	49784	*chunghsingensis*	BAA-1172
Halobacterium		*Methanocaldococcus*	
distributum	51197	*jannaschii*	43067
norici	BAA-852	*Methanogenium*	
salinarum	700922, 17051, 17052, 43214, 33170, 29341, 33171, 19700	*organophilum*	BAA-914
		Methanohalophilus	
Halobaculum		*halophilus*	BAA-1071
gomorrense	700876	*portucalensis*	BAA-912
Halococcus		*Methanocorpusculum*	
morrhuae	17077, 17082	*sinense*	BAA-933
Haloferrarius		*bavaricum*	BAA-929
saponlacus	BAA-1337	*Methanolobus*	
Haloferax		*taylorii*	BAA-911
volcanii	29605	*vulcani*	BAA-932
gibbonsii	33959	*oregonensis*	BAA-928
sulfurifontis	BAA-857	*Methanotorris*	
mediterranei	33500	*formicicus*	BAA-687
sp.	51408	*Methanosarcina*	
sp.	BAA-644	*vacuolata*	35090
Halogeometricum		*siciliae*	BAA-931
borinquense	700274	*mazei*	BAA-159, 43572, 43573, 43340
Halorubrum			
hochstenium	700873	*Methanohalobium*	
lacusprofundi	49239	*evestigatum*	BAA-1072
trapanicum	43102	*Methanospirillum*	
sodomense	33755	*hungatii*	27890
Haloterrigena		*Natrialba*	
turkmenica	51198	*magadii*	43099
Methanobacterium		*Natronococcus*	
ivanovii	BAA-930	*occultus*	43101
alcaliphilum	43379	*Sulfolobus*	
palustre	BAA-1077	*solfataricus*	35092
aarhusense	BAA-828	*Thermococcus*	
Methanobrevibacter		*pacificus*	700653
gottschalkii	BAA-1169	*zilligii*	700529
smithii	35061	*barossii*	BAA-1085
Methanococcus		*Thermoplasma*	
vannielii	35089	*acidophilum*	25905
maripaludis	43000	*volcanium*	51530
aeolicus	BAA-1280		
maripaludis	BAA-1331		
voltae	BAA-1334		
Bacteria			
Desulfovibrio		*Thermoanaerobacter*	
sp.	BAA-1095	*fijiensis*	BAA-1278
Desulfuromonas		*Thiorhodococcus*	
michiganensis	BAA-778	*bheemlicum*	BAA-1362