MICROBIAL CHARACTERIZATION OF JAZAN SABKHA IN SAUDI ARABIA

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Abstract. Prokaryotic diversity of the Jazan sabkha, Saudi Arabia was investigated by using culture dependent techniques and 454 pyrosequencing. While representatives of the *Salinibacteraceae* and *Rubidibacteraceae* families were the predominant groups within bacterial community, comprising the 38.4% and 33.0% of all bacterial OTUs (respectively), archaeal community was dominated by the members of the *Halobacteriaceae* family, constituting 92.8% of all archaeal reads. *Salinibacter* and *Halorhabdus* were the most represented known bacterial and archaeal phylotypes in the community. The novel archaeal phylogroups appeared to be dominant, which made up 71.6% of all archaeal reads. Representatives of the bacterial phyla *Firmicutes*, *Proteobacteria* and *Actinobacteria* were cultured (*Arhodomonas*, *Halobacillus*, *Virgibacillus*, *Halomonas*, *Actinopolyspora*, *Tamilnaduibacter*). Haloarchaeal strains belonging to the genera *Haloferax* and *Halogeometricum* were isolated as well. Diversity and species richness of archaeal assemblages were higher than those of bacterial assemblages, revealed with rarefaction analysis and diversity indices.
Keywords: *Halobacteriaceae, Salinibacter, halophilic bacteria/archaea, pyrosequencing*

Introduction

Halophiles are found in all the three domains of life such as Archaea (e.g., *Halobacterium* sp.), Bacteria (e.g., *Halobacillus* sp.) and Eukaryotes (e.g., green algae, *Dunaliella salina*, brine shrimp, *Artemia franciscana* and halophytic plant, *Atriplex halimus*) (DasSarma and DasSarma, 2012).

"Sabkha" is an Arabic word that is used to express salt-encrusted flat surfaces. Such arid environments are common in the world, e.g. the coast of Baja California (Mexico) and Sinai Peninsula (Egypt) (Akili and Torrance, 1981). There are many coastal sabkhas along the shoreline of the Red Sea and Arabian Gulf, and inland sabkhas in Arabian Peninsula (Powers et al., 1966).

Jazan is a town in southwestern Saudi Arabia bordering the Red Sea and located on a salt dome (Dhowian, 2015). Jazan sabkha belongs to the group of the coastal sabkha which is flooded by seawater and exposed to UV-irradiation, temperature, and desiccation. Hyper saline regions differ from each other in terms of salt concentration, chemical composition and geographical location, which determine the nature of inhabitant microorganisms. These environments are predominantly occupied by both extremely halophilic and halotolerant microorganisms, for example, *Halobacterium* sp., *Haloferax* sp., *Haloarcula* sp., *Halobacillus* sp., *Salinibacter ruber*, *Virgibacillus salarius*, *Bacillus* spp. *and Micrococcus luteus* (Paterekt and Smith, 1985; Arahal et al., 1996; Anton et al., 2002; Solanki and Kothari, 2012; Solomon and Viswalingam, 2013).

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There is an expanding interest for halophilic microbes due to their biotechnological importance as sources of halophilic enzymes, fermentation of soy and fish sauce, biological treatment of saline wastewater and production of β-carotene, compatible solutes, bioplastics and bio fuel (Oren, 2010; Li and Yu, 2012). Therefore revealing the microbial diversity of these hypersaline areas is very important.

The bacterial groups in saline environment be halophilic and halotolerant microscopic organisms of different functionalities i.e sulfur oxidization, phosphate solubilization, cellulose corruption, anti-toxin and compounds creation and so forth (Holguin et al., 2001)

There are several reports on soil properties and constraction problems of sabkhas in Arabian Peninsula, on the other hand microbial compositions were poorly studied (Akili and Torrance, 1981; Baati et al., 2010; Basyoni and Aref, 2015). The aim of this study to reveal prokaryotic assemblage of Jazan sabkha which was analyzed with 16S rRNA gene-based methods.

Materials and Methods

Sampling Area

Sampling was carried out from the surface salt layer of Jazan sabkha (June, 2013). Jazan is a town in southwestern Saudi Arabia bordering the Red Sea and located on a salt dome The study area is located at 16°40′ 00.38″ N and 42°44′ 23.27″ E in south-west of Saudi Arabia. Each sample was collected into 50 ml sterile FALCON tubes (Becton Dickinson) with sterile spatula, and kept at room temperature until use.

Total DNA Extraction and Pyrosequencing

GV filter (0.22-µm pore size, Durapore, Millipore) was used to collect microorganisms. Lysis of the microorganisms were carried out with the addition of the extraction buffer (100 mM Tris-HCl and 100 mM EDTA pH 8.0), lysozyme (3 mg/mL), proteinase K (150 mg/mL) and 10% sodium dodecyl sulfate and the mixture was incubated at 37°C. After the addition of NaCl (5 M) and CTAB solution (10% CTAB, 0.7 M NaCl), the mixture was treated with liquid nitrogen and then put in a water bath (65°C). Purification and precipitation of the DNA were carried out by using phenol-chloroform-isoamyl alcohol (25:24:1) and ethanol. Total DNA was resuspended in Milli-Q water. Pyrosequencing were carried out by ChunLab Inc. (Seoul, South Korea) using Roche 454 GS FLX+/Junior system. Primers targeted the V1-V3 region of the 16S rRNA gene. The reads were assigned into operational taxonomic units (OTUs) at 3% dissimilarity level, by using CD-HIT program and EzTaxon-e database (Kim et al., 2012; Lane et al., 1985; Li et al., 2011). The determination of the community composition and the statistical analyses were carried out using CLcommunity™ program (ChunLab Inc., South Korea).

Cultivation and Sequencing

The sample was serially diluted (original, 10^{-2}, 10^{-4}, 10^{-6}) and inoculated on plates containing 18% and 23% modified growth medium (MGM) (Dyall-Smith, 2009). The incubation was carried out at 37°C for four weeks. Colonies were restreaked on the same medium. The colonies were boiled in Milli-Q water for extracting DNA. 16S rRNA genes of the isolates were amplified by universal primers including 1492r (5'-

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GGTTACCTTGTTACGACTT-3'), 27f (5'-AGAGTTTGATCATGGCTCAG-3') (Lane et al., 1985) and 21f (5'-TTCCGGTTGATCCTGCCGGA-3') (DeLong, 1992). Polymerase chain reactions (PCR) products were digested with *Hinf*I and *Mbo*I restriction enzymes (Fermentas) (37°C, overnight). ARDRA patterns were visualized using 2% agarose gel. Some of the PCR products were purified (Wizard® SV Gel and PCR Clean-Up System; Promega) and sequenced (CEQ^{TM} 8000 DNA sequencer; Beckman Coulter). The sequences were compared with the data in GenBank using BLAST and aligned with MUSCLE program (Edgar, 2004). The phylogenetic trees were constructed by using PhyML (Dereeper et al., 2008). Values of the branch points obtained from 100 bootstrap replicates.

Nucleotide Accession Numbers

The sequences have been deposited in the GenBank database under accession numbers: KJ401317-KJ401326.

Results

Isolated Microorganisms

Viable counts were made on 18% and 23% MGM plates, varied between 1.8 x10^4-4.4 x10^4 cfu/mL. 45 colonies were chosen for further analysis. 16S rRNA genes of the chosen colonies were amplified with polymerase chain reaction (PCR) by using archaea- and bacteria-specific primers. It was determined that 33 of them belonged to Bacteria and 12 of them belonged to Archaea. PCR products of the 16S rRNA genes were analyzed by ARDRA, yielded 2 patterns for Archaea and 8 patterns for Bacteria. The representatives of each pattern were chosen for sequencing revealed that the presence of genera *Haloferax*, *Halogeometricum*, *Arhodomonas*, *Halobacillus*, *Virgibacillus*, *Halomonas*, *Tamilnaduibacter* and *Actinopolyspora*. Phylogenetic analysis of archaeal and bacterial isolates are shown in *Fig. 1* and *Fig. 2*.

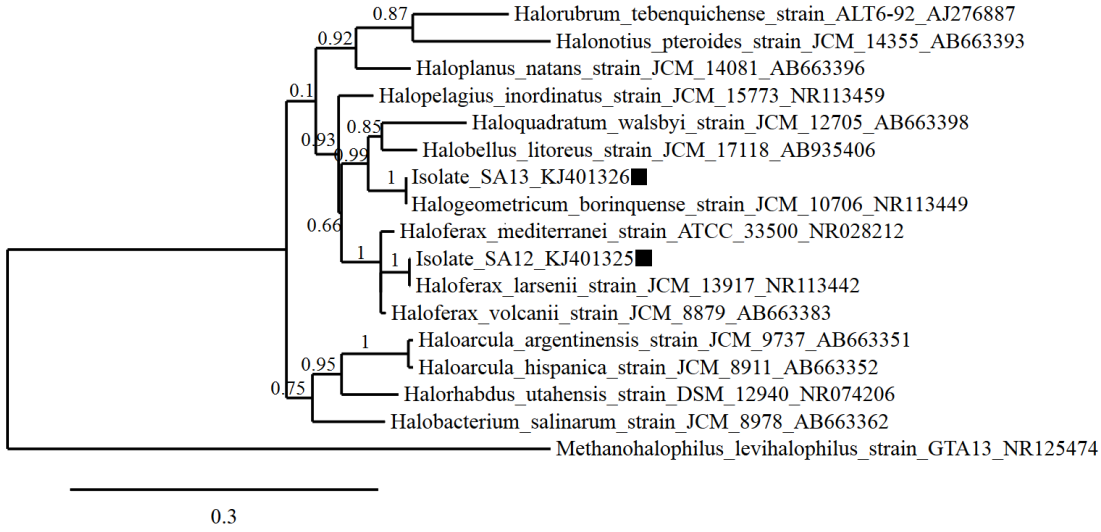

Figure 1. *Maximum-likelihood phylogenetic analysis was performed using PhyML program (http://www.phylogeny.fr). Black squares represent the sequences of the archaeal isolates obtained in this study.*

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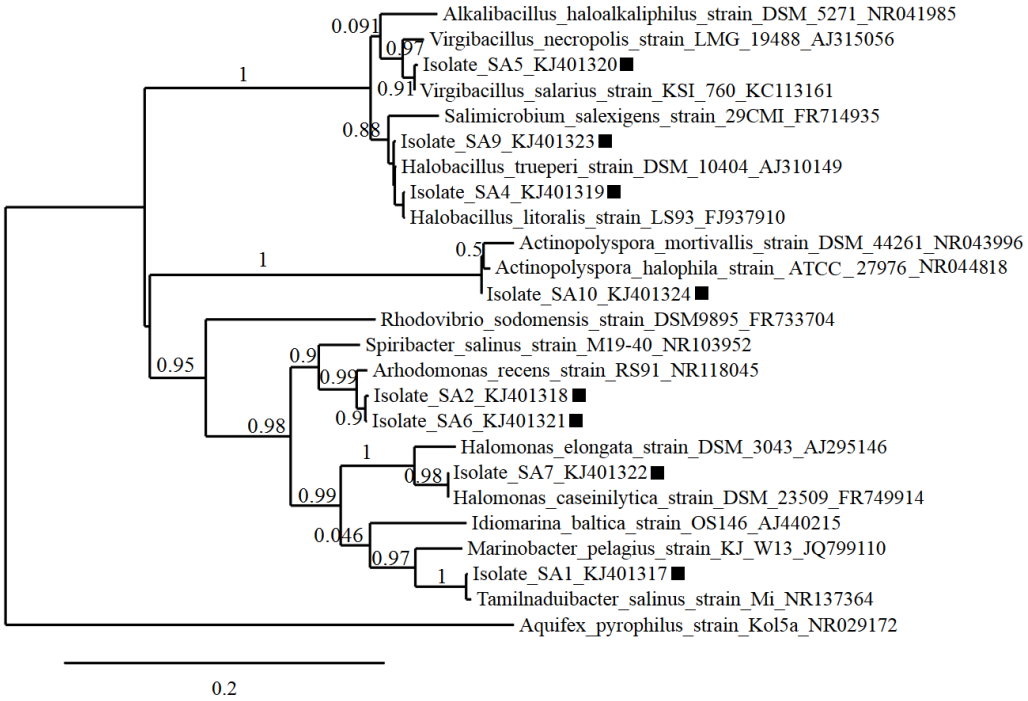

Figure 2. *Maximum-likelihood phylogenetic analysis was performed. Black squares represent the sequences of the bacterial isolates obtained in this study.*

Taxonomic Composition and Statistical Analysis

7183 and 5719 high quality reads were obtained for Archaea and Bacteria (respectively). The number of the archaeal OTUs (1686) were approximately two-fold of the bacterial OTUs (851). The species richness and the diversity of the community were predicted by using Ace and Chao1 estimators and Shannon-Wiener indices, showed that the archaeal populations were more diverse than the bacterial populations (*Fig. 3, Table 1*).

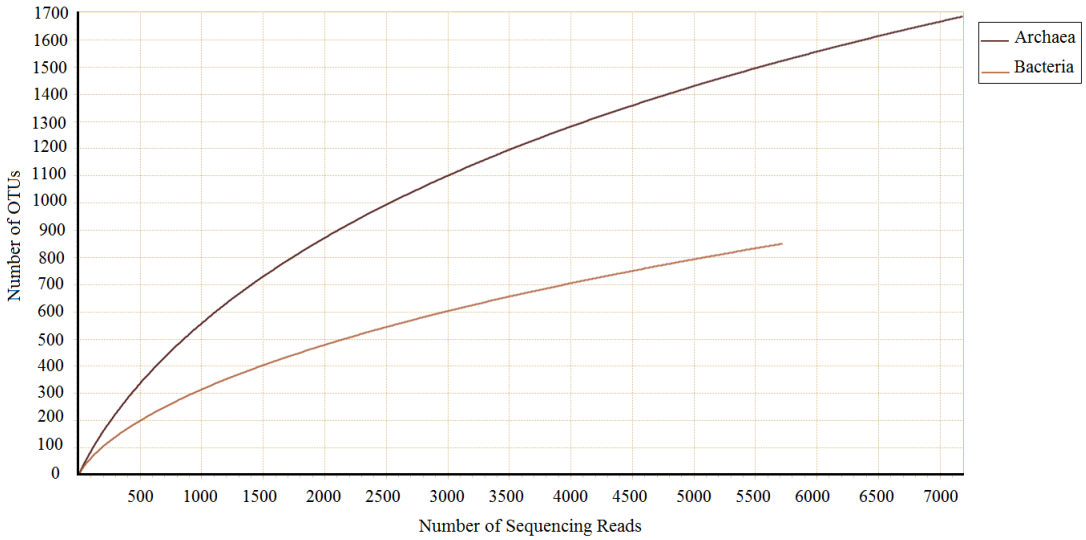

Figure 3. *Rarefaction curves generated from the pyrosequencing reads (cut-off value at 97% similarity).*

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Table 1. *Pyrosequencing data and diversity indices of the archaeal and bacterial populations of Jazan sabkha*

Sample	*Valid reads*	*ARL*	*OTUs*	*Ace*	*Chao1*	*H'*	*Goods Lib. Coverage*
Archaea	*7183*	*426.8*	*1686*	*2589.8*	*2543.2*	*6.61*	*0.90*
Bacteria	*5719*	*401.1*	*851*	*2129.1*	*1572.7*	*5.08*	*0.92*

ARL - Average read length
H' - Shannon-Wiener diversity index

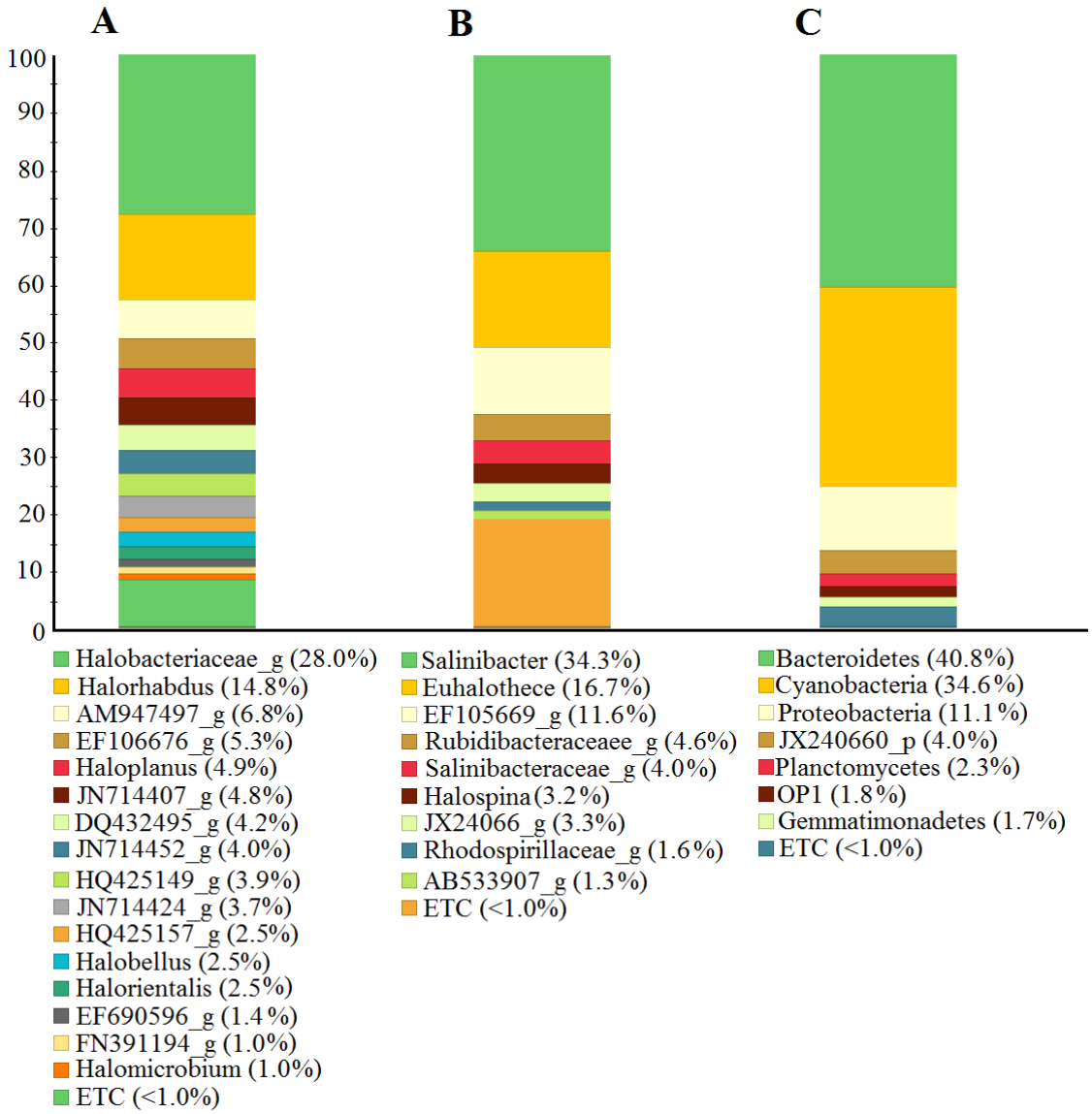

Figure 4. *Bar charts displaying the taxonomic composition for archaeal genera (**A**), bacterial genera (**B**) and bacterial phyla (**C**) (the genera assigned with 94.5% cutoff value).*

The majority of the archaeal OTUs were within the *Euryarchaeota* (99.5% of the archaeal reads) phylum. *Thaumarchaeota* related phylotypes were also detected, but their proportion was very low (0.4%). A novel haloarchaeal phylogroup comprised

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28.0% of the total archaeal community, followed by *Halorhabdus* (14.8%), *Haloplanus* (4.9%), *Halobellus* (2.5%) and *Halorientalis* (2.2%) (*Fig. 4*). The remaining known genera constituted small fraction of the archaeal community, included *Halobacterium*, *Halomicroarcula*, *Halosimplex*, *Salinigranum*, *Salinirubrum*, *Haloferax*, *Halobaculum*, *Salarchaeum*, *Halorussus*, *Natrinema*, *Haloarcula* and *Crenarchaeum*. The most represented bacterial OTUs were within the *Bacteroidetes* (40.8%) and *Cyanobacteria* (34.6%) phyla followed by *Proteobacteria* (11.1%) (*Fig. 4*). *Salinibacter* was the predominant phylotype, which made up 34.3% of the bacterial reads. Most of the cyanobacterial reads were within the *Rubidibacteraceae* family.

Discussion

Bacterial diversity in sabkha ecosystems were investigated previously El Hidri et al. (2013) by using culture-dependent techniques revealed that the isolates were affiliated with *Firmicutes*, *Actinobacteria*, *Proteobacteria* phyla of which representatives also isolated in our study. The strains belong to *Oceanobacillus*, *Virgibacillus*, *Halobacillus*, *Piscibacillus*, *Brevibacillus*, *Salinicoccus*, *Salimicrobium*, *Marinococcus*, *Halomonas*, *Leucobacter*, *Arthrobacter* and *Nesterenkonia* genera were previously isolated from the Sabkhet El Melah and Sabkhet Ennaouel. The strains of *Halobacillus*, *Virgibacillus* and *Halomonas* were also isolated from the Jazan sabkha in our study, of which sequences were closely related with the sequences of the isolates those obtained from different hypersaline environments in China (Li et al., 2011; McKay et al., 2016; Meklat et al., 2011; Mesbah et al., 2007; Montalvo-Rodriguez et al., 1998; Powers et al., 1966; Saralov et al., 2012; Verma et al., 2015). The sequences of the strains of *Actinopolyspora*, *Arhodomonas* and *Tamilnaduibacter* were similar to sequences of the isolates those recovered from the soil (Algeria), brine (Russia) and salt pan (India) (respectively) (Li et al., 2011; Montalvo-Rodriguez et al., 1998; Saralov et al., 2012).

Archaeal isolates belonged to *Haloferax* and *Halogeometricum* genera of which sequences were closely related with the sequences of the isolates previously obtained from the solar salterns in China (Xu et al., 2007) and Puerto Rico (Montalvo-Rodriguez et al., 1998). Very few sequences affiliated with the isolated group detected within the pyrosequencing reads indicated that the cultured species comprised small proportion of the community.

Most of the archaeal reads were assigned to unknown genera, showed similarity with the sequences recovered from the clone libraries from different hypersaline environments in previous studies (Emmerich et al., 2012; Mesbah et al., 2007; Wang et al., 2011). Within the known archaeal genera, *Halorhabdus* phylotypes appeared to be relatively abundant and related sequences were retrieved from the clone libraries of Tunisian solar saltern (Baati et al., 2010).

McKay et al. (2016) investigated microbiota of sabkha in Rub'al Khali (Liwa Oasis, United Arab Emirates) by using 16S rRNA gene cloning, revealed that surface layer of the sabkha mostly composed of phyla *Bacteroidetes* (~63%), *Proteobacteria* (~17%) and *Cyanobacteria* (~3%). We obtained similar results, but cyanobacterial phylotypes were more abundant (~35%) in our study. The most represented bacterial OTUs were closely related with *Salinibacter*, shared similarity with the sequences of the clones those recovered from hypersaline sediment in Death Valley National Park, California (Kim et al., 2012). Cyanobacterial sequences clustered mostly within the *Euhalothece* group, related sequences earlier obtained from the microbial mats of Guerrero Negro,

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Mexico (Harris et al., 2013). The sequences belonging to the *Proteobacteria* phyla were similar to those previously detected in salt plains and hypersaline sediments (Caton and Schneegurt, 2012; Emmerich et al., 2012).

The presence of the representatives of phyla *Euryarchaeota*, *Bacteroidetes*, *Cyanobacteria* and *Proteobacteria* has been demonstrated by this study which focuses on microbial composition of Jazan sabkha.

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