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► **To cite this version:**

S Khelaifia, Didier Raoult. Haloferax massiliensis sp. nov., the first human-associated halophilic archaea. *New Microbes and New Infections*, 2016, 12, pp.96-98. 10.1016/j.nmni.2016.05.007 . hal-01476270

**HAL Id: hal-01476270**

**<https://hal.science/hal-01476270v1>**

Submitted on 9 Jul 2024

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# *Haloferax massiliensis* sp. nov., the first human-associated halophilic archaea

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## Abstract

We report the main characteristics of *Haloferax massiliensis* strain Arc-Hr<sup>T</sup> (= CSUR P974) isolated from stool specimen of a 22-year-old Amazonian obese female patient.

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**Keywords:** Culturomics, genomics, *Haloferax massiliensis*, taxonogenomics, taxonomy

**Original Submission:** 6 May 2016; **Revised Submission:** 9 May 2016; **Accepted:** 10 May 2016

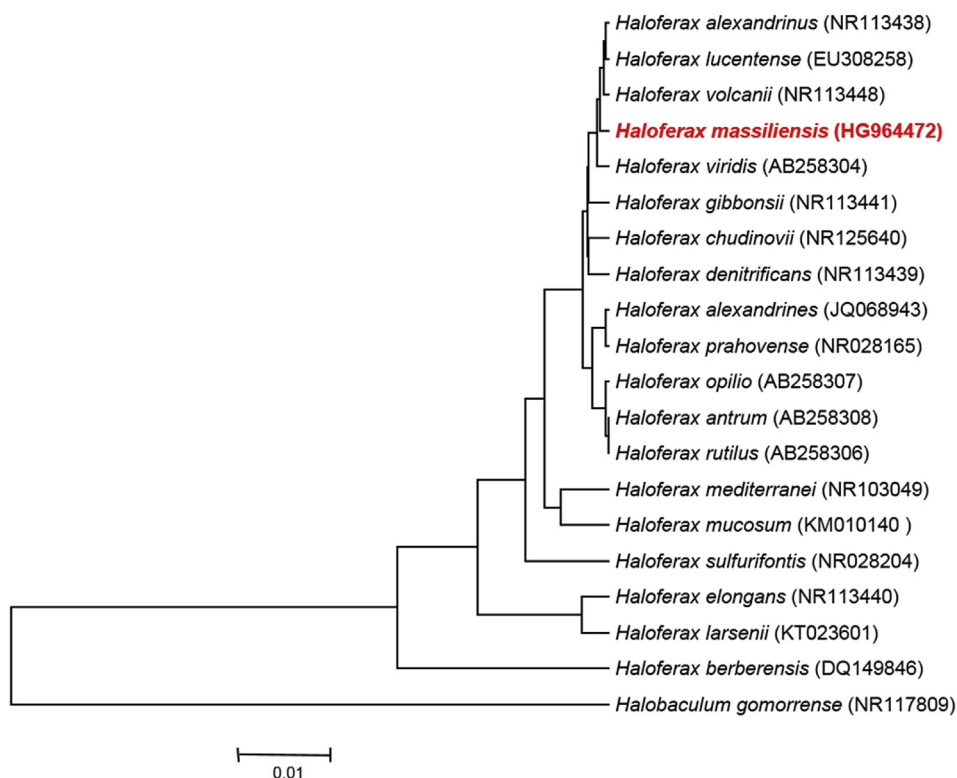
**Article published online:** 14 May 2016

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In December 2013, we successfully isolated the strain Arc-Hr from a stool specimen of a 22-year-old Amazonian obese female patient. Strain Arc-Hr was isolated through the culturomics study, which aims to cultivate and isolate all prokaryotes colonizing the human gut [1]. The patient provided a signed informed consent, and the agreements of the National Ethics Committee of the IFR48 (Marseille, France) were obtained under number 09-022. Strain Arc-Hr was not correctly identified using our matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) on a Microflex spectrometer (Bruker Daltonics, Leipzig, Germany) [2].

The isolate of the strain Arc-Hr was obtained by aerobic culture of the stool specimen into 100 mL of a homemade liquid medium [3] in a flask incubated at 37°C with stirring at

low speed. The pure culture of this halophilic archaea grew aerobically after 7-day incubation at 37°C. Strain Arc-Hr exhibits positive catalase and oxidase activities. The growing colonies on the halophilic medium were red, opaque and 0.5 to 1 mm in diameter. Cells were Gram-negative cocci, nonmotile and non-spore forming with a diameter of 0.9 µm. The 16S rRNA gene was sequenced using the primers as previously described [4] using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France). Strain Arc-Hr exhibited 99.3% sequence similarity with *Haloferax volcanii* JCM 8879 (GenBank accession no. NR113448) [5], the phylogenetically closest validated species with standing in nomenclature (Fig. 1) [6]. Because 16S rRNA gene sequence comparison has been proven to poorly discriminate *Haloferax* species, we sequenced the complete genome of strain Arc-Hr. Genome comparison demonstrated that the draft genome of the strain Arc-Hr is larger than those of *Haloferax volcanii* (NR113448) *Haloferax gibbonsii* (NR113441), *Haloferax lucentense* (EU308258) and *Haloferax alexandrinus* (NR113438) (4.35, 3.9, 3.62, 2.95 and 2.85 Mb respectively). The G+C content of strain Arc-Hr is smaller than those of *H. alexandrinus*, *H. lucentense*, *H. volcanii* and *H. gibbonsii* (65.36, 66, 66.4, 66.6 and 67.1% respectively). The gene content of the strain Arc-Hr is larger than those of *H. alexandrinus*, *H.*



**FIG. 1.** Phylogenetic tree showing position of *Haloferax massiliensis* strain Arc-Hr<sup>T</sup> (= CSUR P974) relative to other phylogenetically close members of family *Haloferacaceae*. GenBank accession numbers are indicated in parentheses. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values ( $\geq 95\%$ ) obtained by repeating analysis 500 times to generate majority consensus tree. Scale bar indicates 1% nucleotide sequence divergence.

*lucentense*, *H. gibbonsii* and *H. volcanii* (3911, 3770, 3593, 2997 and 2917 coding genes, respectively). On the basis of this genome comparison information, we propose to classify the strain Arc-Hr as a new member of the genus *Haloferax* within the family *Haloferacaceae* in the *Euryarchaeota* phylum [7,8], and consequently, we formally propose the creation of *Haloferax massiliensis* sp. nov., with strain Arc-Hr as the type strain of *Haloferax massiliensis* sp. nov.

### MALDI-TOF MS spectrum

The MALDI-TOF MS spectrum of *Haloferax massiliensis* strain Arc-Hr<sup>T</sup> is available at <http://www.mediterranee-infection.com/article.php?leref=256&titre=urms-database>.

### Nucleotide sequence accession number

The 16S rRNA gene sequence was deposited in GenBank under accession number HG964472.

### Deposit in a culture collection

Strain Arc-Hr<sup>T</sup> was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under number CSUR P974.

### Acknowledgement

This study was funded by the Fondation Méditerranée Infection.

### Conflict of Interest

None declared.

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