A korarchaeal genome reveals new insights into the evolution of the Archaea

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Abstract:

The candidate division *Korarchaeota* comprises a group of uncultivated microorganisms which, by their small subunit rRNA phylogeny, may have diverged early from the major archaeal phyla *Crenarchaeota* and *Euryarchaeota*. Here we report the initial characterization of a member of the *Korarchaeota* with the proposed name, "*Candidatus* Korarchaeum cryptofilum" which exhibits an ultra-thin, filamentous morphology. To investigate possible ancestral relationships between deep-branching *Korarchaeota* and other phyla, we used whole-genome shotgun (WGS) sequencing to construct a complete, composite korarchaeal genome from enriched cells. The genome was assembled into a single contig 1.59 Mb in length with a G+C content of 49%. Of the 1602 predicted protein-coding genes, 1382 (86%) could be assigned to a revised set of archaeal COGs. The predicted gene functions suggest that the organism relies on a simple mode of peptide fermentation for carbon and energy and lacks the ability to synthesize *de novo* purines, coenzyme A, and several other cofactors. Phylogenetic analyses based on conserved single genes and concatenated protein sequences positioned the korarchaeote as a deep archaeal lineage with an apparent affinity to the *Crenarchaeota*. However, the predicted gene content revealed that several conserved cellular systems such as cell division, DNA replication, and tRNA maturation resemble the counterparts in the *Euryarchaeota*. In light of the known composition of archaeal genomes, the *Korarchaeota* might have retained a set of cellular features that represents the ancestral archaeal form.

Introduction:

Two established phyla, the *Crenarchaeota* and *Euryarchaeota*, divide the archaeal domain based on fundamental differences in translation, transcription and replication (1). Yet hydrothermal environments have yielded small subunit (SSU) rRNA gene sequences that form deep-branching phylogenetic lineages, which potentially lie outside of these major groups. These uncultivated organisms include members of the *Korarchaeota* (2-4), the Ancient Archaeal Group (5), and the Marine Hydrothermal Vent Group (5, 6). The Nanoarchaeota have also been suggested to hold a basal phylogenetic position (7) but this placement has been debated (8). The *Korarchaeota* comprise the largest group of deep-branching, unclassified archaea and have been detected in several geographically isolated terrestrial and marine thermal environments (2, 4, 5, 9-17).

To gain new insights into the *Korarchaeota*, we revisited the original site where Barns et al. (2) collected the first korarchaeal environmental SSU rDNA sequences (pJP78 and pJP27) from Obsidian Pool, Yellowstone National Park, Wyoming, USA. Continuous enrichment cultures were established at 85° C using a dilute organic medium and sediment samples from Obsidian Pool as an inoculum. The cultivation system supported the stable growth of a mixed community of hyperthermophilic bacteria and archaea including an organism with a SSU rDNA sequence displaying 99% identity to pJP27. The organism was identified as an ultra-thin filament between 0.16-0.18 µm in diameter and variable in length. Whole-genome shotgun (WGS) sequencing was used to assemble an intact composite genome from purified cells originating from the enrichment culture. The complete genome sequence of "*Candidatus* (*Ca.*) Korarchaeum cryptofilum" provides the first look into the biology of these deeply-branching archaea and their evolutionary relationships with *Crenarchaeota* and *Euryarchaeota*.

Results:

Cultivation and *In-situ* Identification. An enrichment culture was inoculated with sediment and hot spring samples taken from Obsidian Pool, YNP. The enrichment was maintained under strictly anaerobic conditions at 85° C, pH 6.5 and continuously fed a dilute organic medium. A stable community of hyperthermophilic archaea and bacteria with a total cell density of approx. $1.0x10^8$ cells/ml was supported for nearly 4 years. Sequences from SSU rDNA clone libraries derived from the enrichment were closely related to other known isolates or environmental sequences from Obsidian Pool [see supporting information (SI) *Text* and SI Fig. 3]. The *Korarchaeota*, were represented by the SSU rDNA clone pOPF_08 which is 99% identical to pJP27 from Obsidian Pool, YNP (2), and pAB5 from Calcite Springs, YNP (9). FISH analysis allowed the positive identification of cells with the pOPF_08 SSU rRNA sequence. Cells hybridizing to Cy3-labeled, *Korarchaeota*-specific probes, KR515R and KR565R, were ultra-thin filaments less than 0.2 µm in diameter with an average length of 15 µm, although cells were observed with lengths up to 100 µm (Fig. 1*A*, SI Fig. 4, SI *Text*).

Cell Preparation and Genome Sequencing. It was observed that filamentous cells hybridizing to probes KR515R/KR565R remained intact in the presence of high concentrations of sodium dodecyl sulphate (SDS; up to 1%) in the hybridization buffer. This feature allowed highly enriched cell preparations to be made by exposing the Obsidian Pool enrichment culture to 0.2% (w/v) SDS (without cell fixation) followed by several washing steps and filtration through 0.45 µm syringe filters. PCR amplified, SSU rDNA sequences from SDS-treated, filtered cell preparations showed that over 99% of the clones sequenced (*n*=180) were identical to the SSU rDNA sequence of pOPF_08 (See SI Fig. 5). Phase contrast (Fig. 1*B*) and electron microscopy (Fig. 1*C*) showed the samples to be highly enriched for ultra-thin filamentous cells with a diameter of 0.16-0.18 µm. DNA clone libraries were constructed from both SDS and non-SDS (libraries BHXI and BFPP respectively) treated enrichment culture filtrates. A total of 23,000 and 11,520 quality sequencing reads from libraries BHXI and BFPP respectively were binned based on %GC content and read depth. Overlapping fosmid sequences containing the pOPF_08 SSU rRNA gene (SI Fig. 6) were used to guide the WGS assembly. Five large scaffolds with a read depth of 8.4-9.9X were closed by PCR (further details are provided in SI *Text*). Single nucleotide polymorphisms occur at a rate of approx. 0.2% across the genome.

General Features. The complete genome consists of a circular chromosome 1,590,757 bp in length with an average G+C content of 49% (Table 1). A single operon was identified that contains genes for the SSU and LSU rRNAs. Forty five tRNAs were identified using tRNAscan-SE (18). A total of 1602 protein coding genes were predicted with an average size of 870 bp. Of the predicted protein coding genes, 73% included AUG; 17%, UUG; and 10% had GUG for start codons. The arCOG (see below) and COG analysis, combined with additional database searches allowed the assignment of a specific biological function to 998 (62%) predicted proteins; for another 246 proteins (15%), biochemical activity but not biological function could be predicted, and for the remaining 358 (22%) proteins, no functional prediction was possible, although many of these are conserved in some other archaea and/or bacteria.

Archaeal COGs. The predicted proteins were assigned to archaeal COGs [(arCOGs) (19) (see SI *Text*, SI Table 3]. Of the 1602 annotated proteins, 1382 (86%) were found to belong to the

arCOGs, a coverage that is slightly lower than the mean coverage of 88% for other archaea and much greater than the lowest coverage obtained for *Nanoarchaeum equitans* (72%) and *Cenarchaeum symbiosum* (58%). When the gene complement was compared to the strictly defined core gene sets for the *Euryarchaeota* and *Crenarchaeota* (i.e., genes that are represented in all sequenced genomes from the respective division, with the possible exception for *C. symbiosum* in the case of the *Crenarchaeota*, but are missing in at least some organisms of the other division), a strong affinity with the *Crenarchaeota* was readily apparent. Specifically, "*Ca.* K. cryptofilum" possesses 169 of the 201 genes from the crenarchaeal core (84%) but only 33 of the 52 genes from the euryarchaeal core (63%). When the core gene sets were defined more liberally, i.e., as genes that are present in more than two thirds of the genomes from one division and absent in the other division, the korarchaeote actually shared more genes with the *Euryarchaeota* than with *Crenarchaeota* (Table 2, SI Table 4). Seven proteins had readily identifiable bacterial but not archaeal orthologs, as determined by assigning proteins to bacterial COGs (20) (SI Table 5). Conceivably, the respective genes were captured via independent horizontal gene transfer (HGT) events from various bacteria. By contrast, there were no proteins specifically shared with eukaryotes, to the exclusion of other archaea. The organism lacks only 5 genes that are represented in all sequenced archaeal genomes, namely, diphthamide synthase subunit DPH2, diphthamide biosynthesis methyltransferase, predicted ATPase of PP-loop superfamily; predicted Zn-ribbon RNA-binding protein, and small-conductance mechanosensitive channel.

Energy Metabolism. The predicted gene set suggests that "*Ca.* K. cryptofilum" grows heterotrophically, using a variety of peptide and amino acid degradation pathways. At least four ABC-type oligopeptide transporters and an OPT-type symporter could import short peptides, which more than a dozen peptidases could hydrolyze into amino acids. As in *Pyrococcus* spp., pyridoxal 5´-phosphate dependent aminotransferases can convert amino acids to 2-oxoacids, while scavenging amines with α-keto-glutarate to form glutamate. Four ferredoxin-dependent oxidoreductases (specific for indolepyruvate, pyruvate, 2-oxoglutarate or 2-oxoisovalerate) could oxidize and decarboxylate the 2-oxoacids, producing acyl-CoA molecules. Four acyl-CoA synthetases can convert this thioester bond energy into phosphoanhydride equivalents. Six aldehyde:ferredoxin oxidoreductase metalloenzymes could oxidize aldehydes derived from these amino acids. Pyruvate could be degraded by this pathway or by a homolog of pyruvate formate lyase. The only terminal reduction reaction predicted from the genome sequence is hydrogen production, apparently catalyzed by two soluble [NiFe]-hydrogenases. An archaeal-type proton-transporting ATP synthase would convert proton motive force produced by anaerobic respiration into ATP. However, in contrast to the system proposed for *Pyrococcus furiosus*, "*Ca.* K. cryptofilum" lacks a membrane-bound proton-translocating hydrogenase (21). Therefore proton translocation must occur through the NADH:quinone oxidoreductase complex or a novel system that might involve homologs of the methanogen *hdrABC*-type heterodisulfide reductase complex. A ferredoxin:NADP oxidoreductase, three flavin reductases, and two electron transfer flavoproteins could mediate electron transfer to the respiratory chain. The korarchaeote also encodes a homolog of a single subunit [Ni-Fe] carbon monoxide dehydrogenase and its accessory proteins in a cluster of methanogen-like genes. Although the physiological role of these proteins in methanogens is unknown, they might confer the ability to oxidize CO produced under anaerobic conditions (22). There is no cytochrome c and no evidence of the dissimilatory reduction of sulfur, sulfite, sulfate, nitrate, nitrite, iron, formate, or oxygen. An abundance of

iron-sulfur proteins, free radical initiating enzymes and the lack of oxidases suggest a strictly anaerobic lifestyle.

Central Metabolism. A partial citric acid cycle is present which includes 2-oxoglutarate: ferredoxin oxidoreductase, succinyl-CoA ligase, succinate dehydrogenase, fumarase, malate dehydrogenase, aconitase, and malic enzyme. These enzymes could be used either for the degradation or for the biosynthesis of glutamate. The organism also encodes the components of a serine hydroxymethyltransferase and glycine cleavage system. One-carbon units from this pterin-dependent pathway are used to produce methionine from homocysteine. The genome encodes few carbohydrate transporters and no hexokinase, although it has a complete pathway for glycolysis from glucose 6-phosphate or for gluconeogenesis. There are no enzymes for the classical or modified Entner-Doudoroff pathways that are found in many *Crenarchaeota*. The organism does have a modified ribulose monophosphate pathway to produce ribose 5-phosphate (23), and a standard pyrimidine biosynthetic pathway. However, it lacks genes for purine nucleotide biosynthesis. Finally, an extensive set of UDP-sugar biosynthesis proteins and glycosyltransferases suggests the presence of glycosylated proteins and lipids. Although "*Ca*. K. cryptofilum" appears to be a proficient peptide degrader, it has an extensive set of amino acid biosynthesis enzymes (see SI *Text*). However, there are many genes missing for coenzyme biosynthesis that are conserved in most of the other *Archaea*. For coenzyme A biosynthesis, it lacks the bifunctional phosphopantothenoylcysteine synthetase/decarboxylase that is found in all other sequenced archaeal genomes except for *Nanoarchaeum equitans* and *Thermofilum pendens* (24). In addition, pathways for riboflavin, pterin, lipoate, porphyrin, and quinone biosynthesis are incomplete.

DNA Replication and Cell Cycle. For initiating chromosome replication, two distinct *orc1/cdc6* homologues and a single minichromosome maintenance protein (MCM) complex are present along with genes encoding single-stranded binding protein (RPA) and primase (PriSL). The genome encodes multiple DNA-dependent, DNA polymerases including 2 family B type enzymes and both the large and small subunits of a euryarchaeal type II polymerase. Genes for the sliding clamp (PCNA), PriSL, and a *gins15* ortholog (25), are clustered with genes for the large subunit of the type II polymerase. A simplified clamp loader complex encodes the large and small subunits of replication factor C. Predicted chromatin-associated proteins include Alba and two H3-H4 histones. Like all known hyperthermophiles, reverse gyrase is present.

  "*Ca*. K. cryptofilum" possesses several genes related to the ParA/MinD family of ATPases involved in chromosome partitioning and SMC-like proteins involved in chromosome segregation. The gene for this ATPase is part of a predicted operon that also includes genes for an FtsK-like ATPase (HerA) and two nucleases, proteins that are thought to comprise the basic machinery for DNA-pumping (26). The organism appears to employ the euryarchaeal mechanism for cell division as indicated by the presence of 7 genes encoding cell division GTPases (FtsZ; SI Fig. 7) (27). One of the *ftsZ* genes is included in a conserved euryarchaeal gene cluster containing *secE*, *nusG*, and several ribosomal protein genes (28, 29). In addition, 5 paralogous *ftsZ* genes are present in a 7-gene cluster that also includes a putative adapter protein (30).

Transcription and Translation. "*Ca*. K. cryptofilum" possesses a full complement of archaeal DNA dependent RNA polymerase (RNAP) subunits. The *rpoA* and *rpoB* genes encoding the

largest subunits of the RNAP are intact. In addition to the typical archaeal RNAP subunits, a coding region of 110 amino acids was identified with limited sequence similarity to the RPB8 of the eukaryotic RNAP. Subsequent in-depth analysis has shown that an ortholog of RPB8, previously thought to be missing in archaea, is also encoded by all sequenced genomes of hyperthermophilic *Crenarchaeota* (31). The RPB8 ortholog resides in a putative operon with the eukaryotic-like transcription factor, TFIIIB. To initiate basal transcription, archaeal homologues for TATA-binding protein, transcription factor B (TFB), and transcription factor E (TFE) are present. Transcriptional regulators are of the bacterial/archaeal type, with the XRE, TrmB, ArsR, PadR-like, CopG, Lrp/AsnC, and MarA families represented in the genome.

The rRNA operon contains a SSU (16S) as well as a LSU (23S) which harbours an intron-encoded LAGLIDADG type homing endonuclease similar to crenarchaeal homologues (32). A total of 33 LSU ribosomal proteins (r-proteins) and 27 SSU r-proteins are present. Notably, r-proteins S30e, S25e, S26e, and L13e that are conserved in the *Crenarchaeota* but are absent in *Euryarchaeota* (33) were identified. In contrast, large subunit r-proteins L20a, L29, and L35ae are missing from the genome.

The tRNA set consists of one initiator tRNA and 45 non-redundant elongator tRNAs. An unusual tRNA^{Ile} with an UAU anticodon is predicted to decode the ATA codon instead of a modified CAU commonly found in archaea (with the exception of *Nanoarchaeum equitans*) (34). Both selenocysteine and pyrrolysine-specific tRNAs are absent. Four tRNA genes contain an intron located one base downstream of the anticodon and one tRNA gene (tRNA^{Ser} CGA) contains an intron in the D-loop. The structural splicing motifs found at all 5 exon-intron junctions and the corresponding homomeric splicing endonuclease appear to reflect the conserved splicing mechanism found in *Euryarchaeota* (35). Also similarly to some *Euryarchaeota*, the universal G-1 residue found at the 5'terminus of tRNA^{His} is not encoded but is predicted to be added posttranscriptionally by a guanylyltransferase. The genome encodes archaeal aminoacyl-tRNA synthetases for all of the amino acids except for glutaminyl-tRNA formation which is mediated via the tRNA-dependent transamidation pathway using the GatD and GatE proteins (36). The LysRS is of the class I type and a homodimeric GlyRS is present. The SerRS is the common type rather than the rarer version found in some methanogens (37). On the other hand, ThrRS appears to be a bacterial type and was likely acquired through a HGT event.

Phylogeny and Evolutionary Genomics. We performed a comprehensive phylogenetic analysis based on combined large and small rRNA subunits, conserved single-gene markers, and conserved concatenated proteins. Collectively, these results demonstrate that "*Ca*. K. cryptofilum" represents a deeply diverged archaeal lineage with affinity to the *Crenarchaeota*. Combined SSU+LSU rRNA subunit trees supported a deep crenarchaeal position (Fig. 2*A*). Likewise, the maximum likelihood based phylogeny of elongation factor 2 (EF2) homologues from archaeal genomes or environmental fosmid sequences corresponded with the rRNA tree (Fig. 2*B*). Phylogenetic analysis of 33 concatenated r-proteins and 3 large RNAP subunits clustered the korarchaeote with *C. symbiosum* in a deep branch joining the hyperthermophilic *Crenarchaeota* (Fig. 2*C*). However, this grouping could be a long branch attraction artifact, and a statistical test showed that a basal position of "*Ca*. K. cryptofilum" identical to that in Fig. 2*A* and 2*B* could not be ruled out. See SI *Text* for separate RNAP subunit and r-proteins phylogenies with compatibility testing.

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Discussion:

Capturing a Korarchaeal Genome. Critical improvements in the cultivation and *in-situ* identification were necessary to resolve a complete korarchaeal genome. The ultra-thin, filamentous organisms hybridizing to *Korarchaeota*-specific probes displayed a thinner and generally longer morphology than what has been previously described for pJP27-type korarchaeote (38). It is not known if the morphological discrepancies are due to differences in the enrichment conditions, hence growth rate, or if variation in cell shape occurs among closely related species. Nevertheless, SDS concentrations that are generally 5 to 50 fold higher than those that are typically required for FISH analyses of hyperthermophilic archaea (39) were necessary for optimal probe penetration. The structural integrity of "*Ca*. K. cryptofilum" in the presence of surfactants is likely attributed to the densely packed S-layer revealed through EM studies (Fig. 1*D*). Exploiting this feature allowed the filamentous cells to be sufficiently purified for WGS sequencing and assembly into a single contiguous chromosome. The proposed name for the organism reflects its elusive nature and reputation for hiding within its community and also for its ultra-thin, filamentous morphology. The proposed genus, *Korarchaeum* gen. nov., stems from the originally proposed phylum designation by Barns *et al.* (3), which is derived from the Greek noun, *koros* or *kore* meaning "young man" or "young woman" respectively; and the Greek adjective *archaios* for "ancient". The proposed species name *cryptofilum* sp. nov., is derived from the Greek adjective, *kryptos,* meaning "hidden" and the Latin noun *filum*, "a thread".

Metabolism. Determining the growth requirements in detail for "*Ca*. K. cryptofilum" was not possible since the organism could only be propagated in a complex enrichment culture. Isolation attempts using Gelrite plates, dilution series, or optical tweezers were unsuccessful. However, the major aspects of the metabolism could be reconstructed from the predicted set of protein-coding genes which suggest an obligately anaerobic, heterotrophic lifestyle with peptides serving as the principal carbon and energy source. In agreement with the predicted metabolism, the enrichment culture was supplied with peptone and traces of yeast extract as the primary carbon and energy source under strictly anaerobic conditions. Anaerobic peptide utilization is a common metabolic strategy among hyperthermophilic *Crenarchaeota* and *Euryarchaeota* (40) and has been characterized in detail in the model organism *Pyrococcus furiosus* (41-44). However, "*Ca*. K. cryptofilum" apparently differs from other known hyperthermophiles in lacking the ability to utilize exogenous electron acceptors such as oxygen, nitrate, iron, or sulfur (45). Protons appear to be the primary acceptor for ferrodoxin-shuttled electrons. To avert possible growth inhibition, removal of molecular hydrogen by flushing with N_2/CO_2 gas and also by possible hydrogen consuming members of the enrichment community such as *Archaeoglobus* and *Thermodesulfobacterium* spp. might have improved cell growth. The organism also appears to lack complete pathways for the *de novo* synthesis of several cofactors which may prevent growth in axenic cultures. These coenzymes must be scavenged from the environment or the organism has evolved alternative modes for producing them. Microbial communities forming high-density mats composed of filamentous cells have yielded the highest number of amplified korarchaeal SSU rDNAs (9-11). Some of the essential nutrients for the growth might be supplied *in-situ* by other mat-forming organisms.

Evolutionary Considerations. Independent phylogenetic analyses based on combined SSU+LSU rRNA, elongation factor 2 (EF-G/EF-2), and concatenated r-proteins + RNAP subunit sequences are compatible with the notion of the *Korarchaeota* being a deeply branching lineage with affinity to the *Crenarchaeota* (Fig. 2). This genome-based assessment corroborates a previous phylogenetic analysis based on a robust set of archaeal environmental SSU rDNA sequences (46). The apparent relationship between the *Korarchaeota* and a member of the marine group 1 *Crenarchaeota* suggested by the phylogeny of concatenated r-protein + RNAP subunits (Fig. 2*C*) is of potential interest. Based on comprehensive phylogenetic analyses and gene content comparisons, the mesophilic *Crenarchaeota* have recently been proposed to form a separate major phylum within the Archaea (47). The apparent affinity between *C. symbiosum* and "*Ca*. K. cryptofilum" presented in our analysis remains to be validated since whole-genome phylogenetic reconstructions are based on a limited number of available archaeal genomes.

The genome revealed a pattern of orthologs that suggests an early divergence within the archaeal domain. The complement of information processing and cell cycle components appears to be a hybrid, with proteins composing the ribosome and RNAP shared, primarily, with *Crenarchaeota*, whereas functions involving tRNA maturation, DNA replication/repair, and cell division being more typical of the *Euryarchaeota* (Table 2). This complex pattern could have resulted from a combination of vertically inherited traits from ancestral organisms supplemented by HGT events. Recent genome analyses have shown that genes believed to be exclusive to the *Euryarchaeota* are also present in some crenarchaeotes. For example, a type-II DNA polymerase and a divergent *ftsZ* homologue are present in *Cenarchaeum symbiosum* (48), and histones are also found in mesophilic and some hyperthermophilic *Crenarchaeota* (49, 50). It remains to be determined whether these genes were vertically inherited from a common archaeal ancestor or were acquired horizontally from members of the *Euryarchaeota* or Bacteria (51).The euryarchaeal type features found in "*Ca*. K. cryptofilum" are generally more similar to those found in thermophilic and hyperthermophilic *Euryarchaeota* (SI Table 6). The presence of several mobile elements in the genome certainly suggests that the gene content may have been influenced by HGT (SI *Text*). Sequencing additional archaeal genomes will aid in determining whether the amalgam of cren-and euryarchaeal characteristics present in the korarchaeal genome represents is an ancient feature or resulted from a combination of HGT and gene loss events. More than a decade after the *Korarchaeota* were introduced based on rDNA sequences (2, 3), identifying "*Ca*. K. cryptofilum" and sequencing its genome has provided a new perspective into the biological diversity of these elusive organisms and the genomic complexity of the archaeal domain.

Materials and Methods:

Sample Collection and Cultivation. Sediment and water samples were collected by B.P.H. from Obsidian Pool, Yellowstone National Park, USA, and ranged from 78°-92° C with a pH of ca. 6.5. The cultivation conditions for the Obsidian Pool enrichment culture were similar to those described previously (38). For details see SI *Text*.

Fluorescence *In-situ* Hybridization Analysis. FISH analysis was performed similar to described previously (39). Cy3-labeled, oligonucleotide probes KR515R (CCAGCCTTACCCTCCCCT) and KR565R (AGTATGCGTGGGAACCCCTC) provided optimal results. The hybridization solution containing 0.9 M NaCl, 0.5% SDS, 100 µg/ml

sheared herring sperm DNA, 0.02 M Tris-HCl (pH 7.2), and 20% formamide (v/v). The wash buffer containing 0.23 M NaCl, 0.1% (w/v) SDS, and 0.02 M Tris-Hcl (pH 7.2). For details see SI *Text*.

Electron Microscopy. Cell pellets were immediately fixed in a solution containing 2.5% glutaraldehyde (EM grade) in 20 mM sodium cacodylate buffer (pH 6.5). EM method details are provided in SI *Text*.

Cell Purification. Fermentor effluent was collected in sterile 2 l glass bottles. Washed cells were briefly exposed to 0.2% SDS (w/v) and then washed 3 times with PBS (pH 7.2). Cell suspensions were then filtered through 0.45 μm syringe filters (MILLEX HV, Millipore, Ireland) in 25 ml aliquots. The filtrate was centrifuged at 6000 rpm for 30 min. to collect the cells. See SI *Text* for detailed protocol.

Genome Sequencing and Assembly. Library construction, sequencing, and assembly were performed at the Joint Genome Institute, Walnut Creek, CA, USA. See SI *Text*.

Comparative Genomics and Phylogenetic Analyses. The predicted protein-coding genes were compared against those from other genomes available in the Integrated Microbial Genomes (IMG) analysis tool (52) and the National Center for Biotechnology Information database. Archaeal COGs were analyzed using the COGNITOR methods (19, 20, 53). An alignment of concatenated small and large subunit rRNA sequences (SSU+LSU rRNA) was constructed based on their conserved secondary structures and refined by hand. See SI *Text* for detailed information regarding phylogenetic analysis and tree construction.

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Figure legends:

Fig. 1. Microscopy of "*Ca*. K. cryptofilum". (*A*) FISH analysis with *Korarchaeota*-specific Cy3-labeled oligonucleotide probes KR515R/KR565R. The undulated cell shape results from drying of the specimen on agar coated slides prior to hybridization. Scale bar represents 5 µm. (*B*) Phase contrast image of korarchaeal filaments following physical enrichment. Scale bar represents 5 µm. (*C*), Scanning electron micrograph of purified cells. (*D*) Transmission electron micrograph after negative staining with uranyl acetate displaying the paracrystalline S-layer. Cells are flattened which increases their apparent thickness.

Fig. 2. Phylogenetic analysis of "*Ca*. K. cryptofilum". (*A*) Maximum likelihood phylogenetic tree of combined (SSU+LSU) rRNAs rooted with corresponding bacterial sequences. Numbers at the nodes indicate bootstrap support. (*B*) Archaeal phylogeny based on translation elongation factor 2 (EF2) proteins rooted with bacterial homologs. The numbers indicate bootstrap support for PhyML/consensus posterior probability (Phyloblast), an asterisk indicates <50 bootstrap support. Where both values were <50, the branch was collapsed. (*C*) Maximum likelihood tree made from aligned sequences of 33 universally conserved ribosomal proteins and the 3 largest RNA polymerase subunits, RpoA, RpoB, and RpoD. Bootstrap support numbers are given at the nodes as a percentage (*n*=10000). Scale bars represent the average number of substitutions per residue.

Table 1. General features of the "*Ca*. K. cryptofilum" genome

Total number of bases	1590757
Coding density (%)	89
G + C content (%)	49
Total number of predicted genes	1661
Protein coding genes	1602
Average ORF length (bp)	870
rRNA genes[*]	3
tRNA genes	45
Genes assigned to COGs	1401
Genes assigned to arCOGs	1382
Genes with function prediction	998
Genes with biochemical prediction only	246
Genes with unknown function or activity	358

[*]16S, 23S, and 5S rRNA

Table 2. Crenarchaeal and euryarchaeal arCOGs in *"Ca.* **K. cryptofilum***"*

arCOG	Cat.*	Function	Eu‡	Cr§
04447	L	DNA polymerase II, large subunit	27	0
04455	L	DNA polymerase II, small subunit	26	0
00872	L	ERCC4-like helicase	26	0
02610	L	Rec8/ScpA/Scc1-like protein	24	0
02258	L	subunit of RPA complex	20	0
00371	D	Chromosome segregation ATPase, SMC	24	0
02201	D	Cell division GTPase FtsZ	26	0
01013	J	Protein with L13E-like domain	0	11
04327	J	Ribosomal protein S25	0	13
04293	J	Ribosomal protein S30	0	13
04305	J	Ribosomal protein S26	0	13
04271	K	RNA polymerase, subunit RPB8	0	12
00393	K	Membrane-associated transcriptional regulator	0	9

*COG functional categories: L, Replication, recombination and repair; D, Cell cycle control, cell division, chromosome partitioning: J, Translation, ribosomal structure and biogenesis; K, Transcription. ‡Number of euryarchaeal (Eu) genomes containing that arCOG (out of 27 total). §Number of crenarchaeal (Cr) genomes containing that arCOG (13 total).
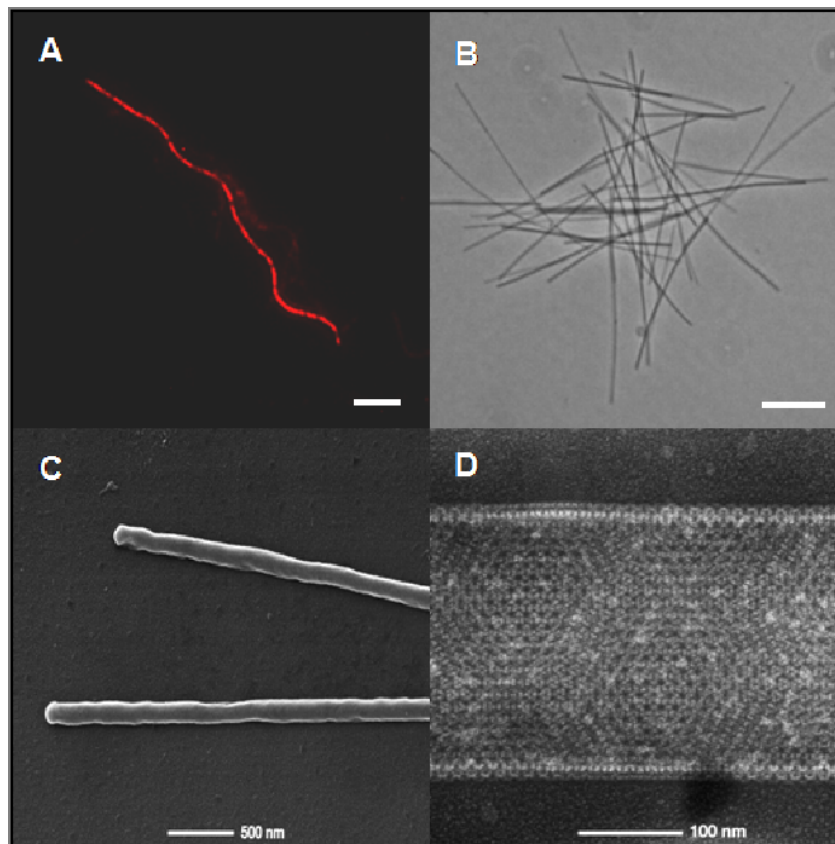
Figure 1.

Figure 2.