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Complete genome sequence of *Crassaminicella* sp. 143-21, isolated from a deep-sea hydrothermal vent

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Running title: Genome analysis of *Crassaminicella* sp. 143-21

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Abbreviations: CDS, coding sequence; COG, clusters of orthologous groups; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; ORF, open reading frame

Abstract

Crassaminicella sp. 143-21, a putative new species isolated from deep-sea hydrothermal vent chimney on the Central Indian Ridge (CIR), is an anaerobic, thermophilic and rod-shaped bacterium belonging to the family *Clostridiaceae*. In this study, we present the complete genome sequence of strain 143-21, comprising 2,756,133 bp with a G+C content of 31.1 %. In total, 2,427 protein coding genes, 121 tRNA genes and 33 rRNA genes were obtained. Genomic analysis of strain 143-21 revealed that numerous genes related to organic matter transport and catabolism, including peptide transport, amino acid transport, saccharide transport, ethanolamine transport and corresponding metabolic pathways. Further, the genome contains a large proportion of genes involved in translation, ribosomal structure, and signal transduction. These genes might facilitate microbial survival in deep-sea hydrothermal vent environment. The genome of strain 143-21 will be helpful for further understanding its adaptive strategies in the deep-sea hydrothermal vent environment.

Keywords: *Crassaminicella*, Chemoorganotroph, Hydrothermal vent, Adaptation, Complete genome.

1. Introduction

Hydrothermal vents are a typical deep-sea ecosystem that possesses a steep gradient in terms of temperature, redox potential, pH, and substrate concentration when hot venting fluids mix with the surrounding cold sea water [1]. Microorganisms inhabiting such ecosystems are mainly chemolithotrophic, which exploiting the chemical disequilibrium provided by the mixing of reducing hydrothermal fluids and oxidizing seawater, and harnessing this energy to fix inorganic carbon into biomass [2, 3]. These microbial communities are important primary producers and fuel the vent ecosystems. Culture-dependent and culture-independent approaches have exposed a vast diversity of chemotrophic microorganisms in hydrothermal vent ecosystems [3-6], however, only a few of them are culturable [7]. Most of investigations of deep-sea hydrothermal vents focused on thermophilic and hyperthermophilic microorganisms, only a few number of moderate thermophiles were isolated [8]. Therefore, these cultivatable moderate thermophiles are likely to provide us with valuable insights into life in the deep-sea hydrothermal systems.

The phylum *Firmicutes* includes a very heterogeneous assemblage of bacteria, some of them were identified as dominant groups in the deep-sea hydrothermal vents [9, 10]. The genus *Crassaminicella* belongs to the *Clostridiaceae* family within phylum *Firmicutes* and was first reported in 2015 [11]. Members of this genus are often obligate anaerobes and heterotrophs that use organic matter such as tryptone and glucose [11, 12]. At the time of writing, this genus comprises only one species of hydrothermal origin, *Crassaminicella profunda* Ra1766H^{T}, which was isolated from

geothermal sediments of the Guaymas Basin at a depth of 2,002 m [11]. Recently, we have isolated a potential new species of genus *Crassaminicella*, strain SY095, from a deep-sea hydrothermal vent on the Southwest Indian Ridge. Genomic analysis showed that strain SY095 genome harbors multiple prophages, some of them carry additional genes that may be involved in the regulation of sporulation [12]. The genome information expanded our understanding of the adaptation strategies of genus *Crassaminicella* to the deep-sea hydrothermal vent high pressure and high temperature environments. In this study, strain 143-21 was isolated from a chimney sample that was collected at the depth of 2,440 m from the site Kali (25.32° S, 70.04° E) located in the *Kairei* hydrothermal field of the intermediate spreading Central Indian Ridge (CIR) using the manned submersible "*Shen Hai Yong Shi*" during the TS10-3 cruise of R/V "*Tan Suo Yi Hao*" on February 2019. Physiological analysis showed that strain 143-21 is obligate anaerobic and heterotrophic moderate thermophiles with an optimum growth temperature of 50 ℃. 16S rRNA gene sequence identities showed that it was most closely related to the *C. profunda* Ra1766H^T (95.91 % identity), which indicated that strain 143-21 represent a novel species within the genus *Crassaminicella*. Here we report the complete genome sequence of strain 143-21 for facilitating an understanding of microbial adaptation to deep-sea hydrothermal vent habitats.

2. Data description

Strain 143-21 grown on JCM 909 medium (https://jcm.brc.riken.jp) at 50 ℃ was harvested in late exponential-phase by centrifugation (4,500 g, 20 min). Genomic

DNA was extracted using a MagAttract DNA Kit (Qiagen, USA) according to the manufacturer's instructions and sequenced on the PromethION and MGI-SEQ 2000 systems at Wuhan Nextomics Biosciences Co., Ltd. (Wuhan, China). About 2.5 Gb clean data were generated to reach approximately 837-fold depth of coverage. Clean reads were assembled using the Unicycler version 0.4.8 [13] and polished using Pilon v1.22 [14]. Gene predictions were made using Prodigal version 2.6.3 [15]. Gene annotation was performed by a sequence similarity search against the non-redundant protein database available from the National Center for Biotechnology Information, Clusters of Orthologous Groups (COG) database [16] and Gene Ontology (GO) database [17]. The carbohydrate-active enzymes (CAZymes) were predicted by the dbCAN meta server [18] using the CAZy database [19]. Metabolic pathways were predicted by using KEGG Automatic Annotation Server (KAAS) [20]. The rRNA and tRNA genes were identified using RNAmer version 1.2 [21] and tRNAscan-SE version 2.0 [22], respectively. Clustered regularly interspaced short palindromic repeat (CRISPR) arrays were analyzed using the Minced program version 0.4.2 [23], and genomic island was identified using Islander program version 1.2 [24]. The graphic circular map of the genome was generated using Circos version 0.69 [25].

Table 1. General features and genome sequencing project information for *Crassaminicella* sp. 143-21 according to MIGS recommendations

Item	**Description**
MIGS data	
Project_name	*Crassaminicella* sp. 143-21 genome sequencing and assembly
NCBI BioProject	Accession: PRJNA743153
NCBI BioSample	Accession: SAMN19999416

Geographic location	Indian Ocean
Latitude and longitude	25.32° S, 70.04° E
Water depth	2,440 m
Collection date	02–2019
Environment (biome)	Ocean biome ENVO:01000048
Environment (feature)	Marine hydrothermal vent ENVO:01000122
Environment (material)	Marine hydrothermal vent chimney ENVO:01000129
Observed biotic relationship	Free living
Trophic_level	Chemoheterotroph
Sequencing method	Oxford Nanopore PromethION and MGI-SEQ 2000
Assembly method	Unicycler version 0.4.8
Coverage	837.0x
Finishing_strategy	Complete
General feature	
Classification	Domain *Bacteria* Phylum *Firmicutes* Class *Clostridia* Order *Clostridiales* Family *Clostridiaceae* Genus *Crassaminicella*
Gram stain	Positive
Cell shape	Rod
Motility	Motile
Relationship to oxygen	Obligate anaerobe
Optimal temperature	50 ℃
Genomic features	
Size (bp)	2,756,133
G+C content (%)	31.1
Number of predicted CDSs	2,427
Genes assigned to COG	1,991
Genes assigned to KEGG	1,355
Number of rRNA genes	11, 11, 11 (5S, 16S, 23S)
Number of tRNAs	121
Number of CRISPR Arrays	4

The resulting complete genome of *Crassaminicella* sp. 143-21 consists of a single circular chromosome comprising 2,756,133 nucleotides with 31.1 % G+C content (Fig. 1; Table 1). No plasmid was identified in the genome. A total of 2,427 protein-coding sequences (CDS) were predicted, which covered approximately 85.59 % of the genome. The genome encodes 121 tRNA genes, 33 rRNA genes, and 4 loci of

CRISPR. Among the 2,427 CDSs, over half CDSs were annotated using the COG (82.04 %), GO (64.56 %), and KEGG (55.83 %) databases. Upon COG classification, 1,991 genes were assigned to 23 functional categories (Fig S1), whereas the remaining 436 genes could not be assigned to any category. The abundance of COG category in strain 143-21, such as cell cycle control, cell division, chromosome partitioning (D), Coenzyme transport and metabolism (H), translation, ribosomal structure and biogenesis (J), replication ,recombination and repair (L), and mobilome: prophages, transposons (X), are higher than that in *Crassaminicella* sp. SY095 and *C. profunda* Ra1766H (Fig S1). While other categories, including amino acid transport and metabolism (E), carbohydrate transport and metabolism (G), inorganic ion transport and metabolism (P), and secondary metabolites biosynthesis, transport and catabolism (Q), are lower than the other two strains. These differences may reflect the adaptation of strains to its local habitat.

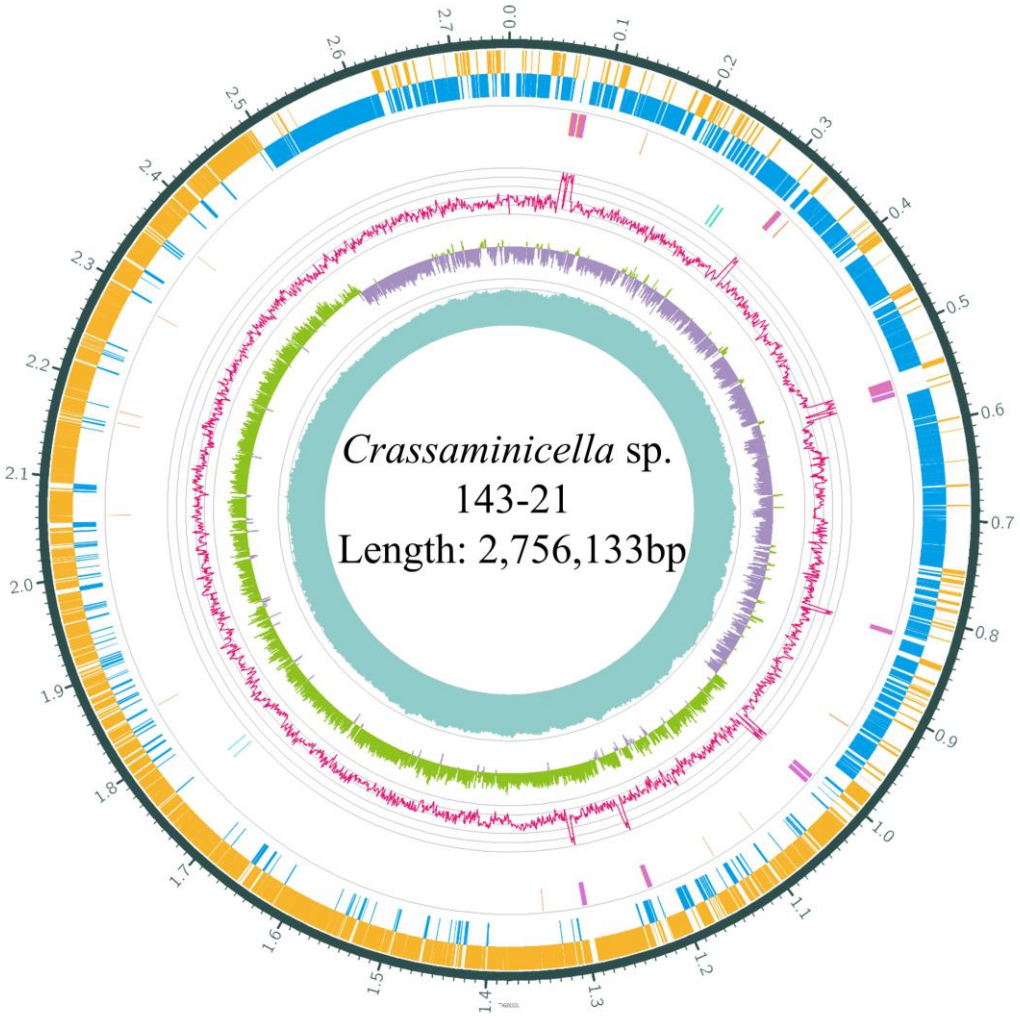


Fig. 1. Schematic representation of the *Crassaminicella* sp. 143-21 genome. Labeling from the outside to the center is as follows: circle 1, genes on the forward strand; circle 2, genes on reverse strand; circle 3, RNA genes (tRNAs orange, rRNAs purple); circle 4, CRISPR arrays; circle 5, GC content; circle 6, GC skew; and circle 7, sequencing depth.

2.1 Organic matter catabolism and energy metabolism

Strain 143-21 is chemoheterotrophic that can grow on amino acids, tryptone and saccharides. Correspondingly, its genome encoded for several transport systems that might participate in the uptake of peptides, amino acids, glucose, ribose, fructose, and raffinose/stachyose/melibiose. We further analyzed the utilization pattern of carbon

source with the Carbohydrate-Active Enzymes (CAZy) database. The result showed that strain 143-21 genome contains 35 CAZymes including 18 glycosyltransferases (GTs), 10 glycoside hydrolases (GHs), 6 Carbohydrate esterases (CEs), and 1 carbohydrate-binding modules (CBM). GTs could catalyze glycosyl group transfer to a nucleophilic group and form a glycoside, which may be involved in the amino sugar and nucleotide sugar metabolism. Among GHs, genes related to degradation of N-acetylglucosamine, glycogen, cellobiose, maltose, and fructose were found. We speculated that these genes could be helpful for the survival of strain 143-21 in deep-sea vent habitats by reinforcing acquisition of nutrients from the extreme environment. Strain 143-21 contained a great number of genes that encoded complete glycolysis/gluconeogenesis pathway and pentose phosphate pathway. In addition, 12 families of peptidases and 10 kinds of aminotransferases were annotated, which digest peptides to produce a variety of amino acids and further metabolism.

In the genome of strain 143-21, thirty genes encoding ethanolamine utilization-related proteins were observed in two clusters (Fig. 2). Gene cluster I (KVH43_01845-01895) contains the key genes of ethanolamine utilization, *eutB* and *eutC*, which encoding the two subunits of the ethanolamine ammonia lyase and breaks ethanolamine down into the gases acetaldehyde and ammonia [26]. In addition, some accessory genes and regulatory genes can be found in this cluster. Gene cluster II (KVH43_06710-06800) mainly contains genes encoding the bacterial microcompartments, where the catabolic reactions occurred efficiently [26]. Interestingly, such gene clusters were also presented in the strain SY095 genome,

while absent in the genome of strain Ra1766H. All microbial cells are made of a bilayer of phospholipids, which contain the membrane lipid phosphatidylethanolamine. Ethanolamine can be produced when phosphodiesterases break down phosphatidylethanolamine, and is considered a valuable source of carbon and/or nitrogen for bacteria capable of its catabolism [26]. Deep-sea vent ecosystem is the vast reservoir of microorganisms, it is plausible that cell membrane decomposition will accompany the release of ethanolamine. Thus, possessing ethanolamine utilization genes provide substantial competitive advantages for microbes surviving in such extreme environment.

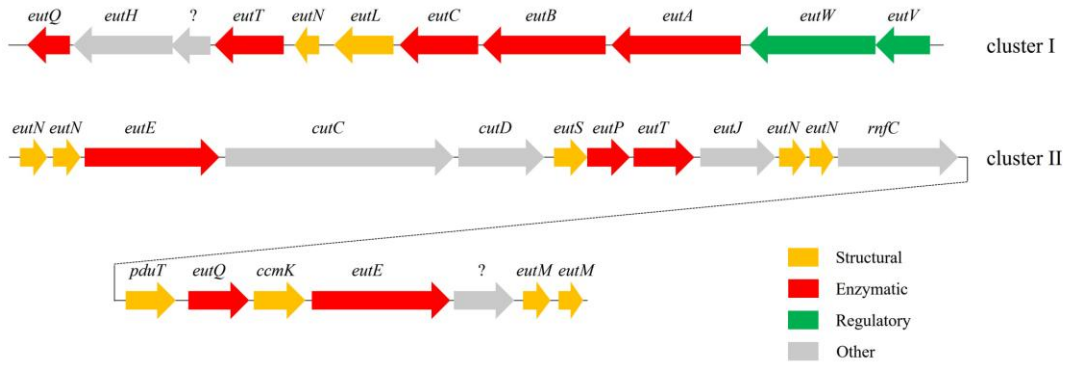


Fig. 2. The putative ethanolamine utilization gene clusters and gene organization of the strain 143-21.

Genomic analysis also predicted an electron transport complex encoding gene cluster, which is assumed to encode a membrane-bound enzyme complex with six subunits (RnfCDGEAB). Rnf complex is a respiratory enzyme that oxidizes reduced ferredoxin and reduces NAD, coupled to ion (Na^+) transport across the cytoplasmic membrane and generate an electrochemical gradient for ATP synthesis [27, 28]. Moreover, ten genes encoding F0F1 ATP synthase were observed in strain 143-21 genome. Intriguingly, we also identified similar gene clusters encoding Rnf complex

and F0F1 ATP synthase in the genomes of strain SY095 and strain Ra1766H. It is proposed that Rnf complex coupled with F0F1 ATP synthase form a simple respiratory chain to increase the ATP yield, which could help *Crassaminicella* species to survive in the deep-sea hydrothermal vent environment.

2.2 Environmental adaptation

Genomic analysis showed that strain 143-21 possesses a series of genes related to high pressure and high temperature adaptation. Firstly, according to the COG categorization, strain 143-21 genome contains a large proportion of genes (10.20 %) related to translation, ribosomal structure and biogenesis. It has been reported that increasing the synthesis of ribosome subunits and tRNA synthase could help vent microorganism to counteract the effects of HHP on the ribosome complex [29]. Secondly, strain 143-21 genome contains 9.84 % of total COG related to signal transduction mechanisms, which is 7.76 % in *Crassaminicella* sp. SY095 and 10.88 % in *C. profunda* Ra1766H (Fig S1). For strain 143-21, twelve histidine kinase sensor and eleven diguanylate cyclase were identified. Histidine kinase acts as a sensor in a two-component signal transduction system, diguanylate cyclase are involved in the synthesis of global second messenger cyclic diguanosine monophosphate, which involved in many aspects, such as biofilm formation, motility, and resistance [30]. As reported, two-component regulatory systems are crucial for strains to sense changes of surrounding environment [31]. Thus, it is reasonable to suppose that possessing multiple signal transduction systems plays a role in microbial survival in deep-sea hydrothermal vent environment. Thirdly, strain 143-21 can form an endospore at late

stage of growth. Searching its genome, we discovered that a total of 73 genes are involved in sporulation initiation, sporulation regulation, DNA translocation, engulfment, cortex synthesis, coat formation and assembly. *spo0A* is the key gene for sporulation initiation, which was activated after phosphorylated by histidine kinases. Once Spo0A was phosphorylated, many genes will participate in the sporulation regulation, such as *spoIIAA*, *spoIIAB*, *spoIIE*, *spoIIGA*, *spoIIM*, *spoIIR*, *sigE-H*, *spoIIIA-H*, and *spoIVA-B*. At spore assembly stage, SpoVM functions as a landmark protein. In addition, there are also 21 genes associated with spore germination. Forming an endospore under extreme conditions and spore germination in suitable environments may help strain 143-21 surviving in the changing deep-sea hydrothermal vent environment. Fourthly, strain 143-21 genome harbor 89 transposases, which may facilitate genomic rearrangements and gene duplication. A high proportion of transposases confer strain a strong genetic plasticity to deal with changing vent environment. In addition, the strain 143-21 genome contains four CRISPR arrays comprising a total of 78 spacer sequences, significantly more than the spacers identified in strain SY095 (28) and strain Ra1766H^T (53). Variation in CRISPR array sequences among different *Crassaminicella* species is probably an adaptation to the local habitats. In conclusion, the genomic analysis has revealed the genetic basis of strain 143-21 and provided insight into the adaptation strategies used by *Crassaminicella* spp. in the deep-sea hydrothermal vent.

2.3. Genome sequence accession numbers

The complete genome sequence of *Crassaminicella* sp. 143-21 was deposited at

GenBank database under the accession number CP078093. The strain has been submitted to Marine Culture Collection of China, MCCC (http://www.mccc.org.cn/) with accession number MCCC 1K06400.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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