Complete genome sequence of Acidithiobacillus ferrooxidans YNTRS-40, a strain of the ferrous iron- and sulfur-oxidizing acidophile

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Short report

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Abstract

Acidithiobacillus ferrooxidans YNTRS-40 (A. ferrooxidans) is a chemolithoautotrophic aerobic bacterium isolated from Tengchong, Yunnan Province, China and with a broad growth pH range of 1.0-4.5. This study reported the genome sequence of this strain and the information of genes related to the adaptation of diverse stresses and the oxidation of ferrous iron and sulfur. Results showed that YNTRS-40 possesses chromosomal DNA (3,209,933-bp) and plasmid DNA (47,104-bp). The complete genome of 3,257,037-bp was consist of 3349 CDS genes comprising 6 rRNAs, 52 tRNAs and 6 ncRNAs. There are many encoded genes associated with diverse stresses adaptation and ferrous iron and sulfur oxidation such as rus operon, res operon, pet Ⅰ, pet Ⅱ, sqr gene, doxDA , cydAB and cyoABCD . This study will provide essential information for further application of A. ferrooxidans YNTRS-40 in industry.

Introduction

Acidithiobacillus usually found in acidic environments with heavy metal and oligotrophic conditions. The members of this genus contribute to the formation of acidic habitats, the acidification of waters [1, 2], and the biogeochemical cycle of iron and/or sulfur [3, 4]. The *Acidithiobacillus* genus contains seven validated species, including *A. ferrooxidans* [5], *A. ferriphilus* [6], *A. ferrivorans* [7], *A. ferridurans* [8], *A. albertensis* [9], *A. thiooxidans* [10], and *A. caldus [*11]. The former four dominant species can obtain energy for growth by using ferrous iron, elemental sulfur, reduced sulfur compounds, hydrogen or tetrathionate as electron donors [6, 8, 12–14].

A. ferrooxidans has iron- and sulfur-oxidizing abilities and can grow in the environments with high concentrations of metal ions such as pyritic ore bodies, coaldeposits and their acidified drainages [15–20]. Therefore, it has potential utilization in microbial electrosynthesis systems, eco-friendly bioleaching technology, biological desulfurization and machining of workpiece. Until now, nine *A. ferrooxidans* strain genomes (ATCC 23270, ATCC 53993, Hel18, BY0502, CCM 4253, IO–2C, YQH–1, DLC–5 and RVS1) have been available in the public databases. The genomes of these strains only reported chromosome genomes, and the chromosome genomes and plasmid genomes were not detected at the same time. Additionally, only strains ATCC 23270 and ATCC 53993 were complete genome sequence [20–22]. The genome of strain YNTRS–40 reported in this paper was complete and contained a plasmid, which is the first report on the simultaneous acquisition of chromosome genome and plasmid genome. The plasmid genome could be helpful in studying the metabolic characteristics of these microbes.

To further investigate the genetic characteristics and application potentialof *A. ferrooxidans* YNTRS–40, its genome was entirely sequenced and annotated. Our findings would be useful for understanding the roles and potential of *A. ferrooxidans* in the field of geobiology, biomedicine, and technology.

Methods

Growth conditions, genomic DNA isolation and morphological detection

A. ferrooxidans YNTRS–40 was isolated from the soil soaked in drainage water in Tengchong, Yunnan Province, China. General features of *A. ferrooxidans* YNTRS–40 are showd in Additional file 1: Table S1. This strain grown in modified 9K medium containing 2.4 g $(NH_4)_2SO_4$, 0.1 g KCl, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$ and 0.01 g $Ca(NO_3)_2$, 40 g $FeSO_4 \cdot 7H_2O$ per liter water with pH 1.75 and incubated at 28^oC for 48 h with agitation at 120 rpm on a shaker. Genomic DNA was obtained using Sodium Dodecyl Sulfate (SDS) method and Blood & Cell Culture DNA Midi Kit (QIAGEN, Germany) following the manufacturer's standard protocol. Cell morphology of *A. ferrooxidans* YNTRS–40 was detected using scanning electron microscopy (Hitachi S–4800, Japan) (Fig. 1).

Genome sequencing, assembly and annotation

The sequencing of the complete genome were performed on Nanopore GridION X5 (Oxford Nanopore Technologies, United Kingdom) [23] by constructing 1D genomic DNA library using the Ligation Sequencing Kit 1D (Oxford Nanopore Technologies, United Kingdom). After quality filtering, the high-quality reads were assembled into contigs using the Canu v1.7.11 [24] and the assembled data was optimized by using Pilon v1.22 [25]. All sequencing project information were displayed in Additional file 1: Table S2.

Coding sequences (CDS) in the genome were predicted through Prodigal v2.6.3 [26]. The tRNAs and rRNAs of the genome were predicted by tRNAscan-SE v2.0 [27] and RNAmmer v1.2 [28], respectively. The genome sequence was annotated by the Rapid Annotation Subsystem Technology (RAST) [29], the Kyoto Encyclopedia of Genes and Genomes (KEGG) [30], the Clusters of Orthologous Groups (COG) [31] and the Gene Ontology (GO) [32]. The circular map of the genome was obtained using Circos v1.7.11 (Fig. 2) [33].

Based on 16S rRNA gene sequence of strain YNTRS–40 (Accession number: MK811409) and other members of *Acidithiobacillus* obtained from GeneBank, phylogenetic analysis was carried out by MEGA5.05 software [34] using CLUSTAL W to perform a multiple alignment [35] and the phylogenetic tree were constructed by the neighbor joining method (Fig. 3) [36].

Results And Discussion

A. ferrooxidans YNTRS–40 is a gram-negative non-endospore-forming chemolithoautotrophic aerobic bacteria in the order *Acidithiobacillales* of the class *Acidithiobacillia* (Additional file 1: Table S1). It possesses resistance against heavy metal and oligotrophic conditions. Although several *Acidithiobacillus* species with the capacity of iron and sulfur oxidization were identified from acidic environments, their genetic features associated with the resistance to extreme environment were ambiguous [4]. In this study,

A. ferrooxidans YNTRS–40 was isolated and its genome was sequenced to analyze the stress resistance. Microscopically, YNTRS–40 cells displayed rod-shaped and were 0.28–0.40 μm in width and 1.00–1.68 μm in length (Fig. 1). This strain grew to a logarithmic stage fastly after 48 hours under aerobic conditions at pH 1.75, 28^{o}C and 120 rpm in a shaker with modified 9K medium. The complete genome sequences have been submitted to GeneBank under the accession number CP040511 (Chromosome) and CP040512 (Plasmid).

The genome size of *A. ferrooxidans* YNTRS–40 is 3,257,037-bp, and the genome contains one circular chromosome of 3,209,933-bp with 58.54% GC content and one circular plasmid (47,104-bp with 56.43% GC content). The circular chromosome comprised 3349 predicted CDS genes, 6 rRNAs, 52 tRNAs and 6 ncRNAs (Table 1) and the circular plasmid contained 70 predicted CDS genes. The statistics and properties of the genome were summarized in Table 1. Total 2015 genes identified from chromosome were classified into 26 functional categories based on Cluster of Orthologous Groups (COG) (Table 2) [37]. Among all categories, the inorganic ion transport and metabolism category (P, 6.70%), the energy production and conversion category (C, 6.40%) and the defense mechanisms category (V, 4.47%) indicated that the strain YNTRS–40 can grow in the environment with high concentrations of metal ion.

Based on 16S rRNA gene sequence analysis, it can be seen that all strains clustered separately into different clades, such as *A. ferriphilus* (Clade □), *A. ferrivorans* (Clade □), *A. ferridurans* (Clade □), *A. ferrooxidans* (Clade □), *A. thiooxidans* (Clade □), *A. albertensis* (Clade □), and *A. caldus* (Clade □). This finding was similar to a study by Zhang et al. [38] and slightly different from a literature, in which *A. ferridurans, A. thiooxidans* and *A. albertensis* clustered into the common clade [14]. The strain YNTRS–40 appeared to represent a coherent group with *Acidithiobacillus ferrooxidans* ATCC 11821 and *Acidithiobacillus ferrooxidans* ATCC 53993 (Fig. 3).

Genomic features related to adaptation to diverse stresses

Acidithiobacillus spp. possess extreme environmental resistance, and they can adjust to extremely acidic conditions (grow optimally at pH 2.0 and survive in pH 1.0–4.5) and their survival, colonization, growth and development [39, 40]. To balance the extracellular and intracellular environment heterogeneity in extremely acidic habitats containing heavy metal ions, these acidophilic microorganisms diverge and evolve to possess the acid and metal resistance [38].

Genome analysis using the Rpsblast and the Interproscan v5.30–69.0 [41] revealed that several functional genes involved in the adaptation of strain YNTRS–40 to extreme environments (Table 2 and Additional file 1: Table S3). The expression of proteins such as oxidoreductase and transferase in the cell should be regulated under the environment containing sulfur and metal ions. It has been documented that the oxidoreductase and transferase not only participate in energy generation but also enhance tolerance to environmental stress [42, 43]. Based on the category of biological process in Additional file 1:

Table S3, the gene function of strain YNTRS–40 such as response to extracellular stimulus, cellular response to stress and response to oxidative stress indicated that it could cope with extreme environmental stress.

Based on the COG analysis (Table 2), the functional genes related to defense mechanisms (V), cell wall/membrane/envelope biogenesis (M), amino acid transport and metabolism (E), inorganic ion transport and metabolism (P) and general function prediction only (R) were slightly more than the other genes. These revealed that this strain exhibited excellent environmental adaptability and has potential applications in ecological industry such as sulfur removal from gases, mental extraction from electronic waste [44]. Additionally, the genes associated with function unknown (S) indicated that strain YNTRS–40 might possess some new genes [45].

The plasmid usually contains the genes related to the secondary metabolism according to the characteristics of microorganisms [46]. There were 70 CDSs in the circular plasmid of strain YNTRS–40, and 39 CDSs of them were predicted as hypothetical proteins, and the rest were found to involve in metabolism and defense (Additional file 1: Table S4). The RAST annotation results showed that the plasmid comprised all kinds of secondary metabolism-related genes, transcriptional regulatory genes, transposase-related genes, mobile element protein-related genes and stress-tolerance genes. These indicated that the primary metabolism-related genes were not present in this plasmid, and the presence of plasmid might favor the adaptation of this strain to environmental stress.

Genomic features related to the oxidation of ferrous iron and sulfur

A. ferrooxidans can gain energy from the oxidation of Fe^{2+} for growth and survival [5, 20]. During the oxidation of Fe^{2+}, most of the electrons are transferred to O_2 along the potential gradient which called downhill potential gradient, while a small part of electrons is transmitted conversely along the potential gradient which named uphill potential gradient [47]. In the latter process, the NAD(P)H is generated and involve in CO_2 fixation and aerobic metabolism [20, 47, 48]. These two electron transfer pathways, namely the downhill and the uphill potential gradient are interrelated [47].

The KEGG analysis showed that several coding genes related to the downhill and the uphill electron transfer pathway existed in the strain YNTRS–40. Among them, the *rus* operon, which is consist of *cyc2, cyc1, cup, coxB, coxA,coxC, coxD* and *rus* genes, were found to involve in electron transfer in the downhill electron pathway [49]. The previous study suggested that the expression and regulation of the *rus* operon are associated with the substrate electron donor in environment [49]. The operon might be activated persistently when bacteria use Fe^{2+} as an electron donor, but only expressed transitorily during the period of early logarithmic growth when cells take S as an electron donor [50, 51]. Additionally, the *pet*, *pet* and *res* operon were found in genome of the strain YNTRS–40. Among them, *pet*encodes a bc_1 complex that

participates in the inverse electron transfer when the strain uses Fe^{2+} as a substrate, and *petI* encodes another bc_1 complex, which is responsible for forwarding electron transfer when S is used as substrate [52]. The *res* operon near the *pet* operon encodes ResB and ResC protein which might serve as a molecular chaperone in the maturation process of the c_1 cytochrome of the bc_1 complex [53].

A. ferrooxidans also can obtain the energy required for growth by oxidizing reduced sulfur. Sulfur was found to be more favorable energy source than Fe^{2+} due to it can provide more ATP than Fe^{2+} at the same molar level [54, 55]. The KEGG results indicated that some genes involved in sulfur oxidation, including *sqr, doxDA, cydAB* and *cyoABCD* genes which code sulfide quinone reductase, thiosulfate quinone oxidoreductase and thiosulfate dehydrogenase, respectively. These genes might be upregulated in strain YNTRS–40 when sulfur is used as substrate. Additionally, the enzymes encoded by these genes were coupled to a respiratory chain and occurred at different nodes in the respiratory chain [56]. These results suggested that the strain YNTRS–40 has potential for industrial application through iron and sulfur oxidizing such as metal bioleaching, gas desulfurization, and bioremediation.

Conclusions

The genome of *A. ferrooxidans* YNTRS–40 revealed that the strain could grow well under extremely acidic conditions containing heavy metal ions and has the ability to remove sulfur from gases and extract metal from solids, since it contained various genes participating in the adaptation to environmental stress and the oxidation of ferrous iron and sulfur. This paper first simultaneously reported the chromosome genome and plasmid genome of *A. ferrooxidans.* It could be helpful to research the metabolic characteristics and commercial application potential of the *A. ferrooxidans* YNTRS–40 in future.

Declarations

Abbreviations

CDS: Coding sequences; MIGS: Minimum information on the genome sequence; SDS: Sodium dodecyl sulfate; RAST: Rapid annotation subsystem technology; KEGG: Kyoto encyclopedia of genes and genomes; COG: Clusters of orthologous groups; GO: Gene ontology; NAD(P)H: Nicotinamide adenine dinucleotide (phosphate); ATP: Adenosine triphosphate

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets generated and/or analysed during the current study are available in the NCBI repository (BioProject number: PRJNA543563).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YZ and LY designed the study, with input from all authors. YN and LY collected samples. SZ and DZ performed the laboratory work (culture, DNA extractions). YZ performed the date processing and bioinformatics analyses. YZ, WW and LY drafted the manuscript. All authors read, improved and approved the final manuscript.

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References

1. Colman DR, Poudel S, Hamilton TL, Havig JR, Selensky MJ, Shock EL, Boyd ES. Geobiological feedbacks and the evolution of *thermoacidophiles*. ISME J. 2018;12(1):225-36.
2. Nuñez H, Moya-Beltrán A, Covarrubias PC, Issotta F, Cárdenas JP, González M, Atavales J, Acuña LG, Johnson DB, Quatrini R. Molecular systematics of the genus *Acidithiobacillus*: insights into the phylogenetic structure and diversification of the taxon. Front Microbiol. 2017;8:30.
3. Hongbo Z, Yisheng Z, Xian Z, Lu Q, Menglin S, Yu Y, Yansheng Z, Jun W, Hyunjung K, Guanzhou Q. The dissolution and passivation mechanism of chalcopyrite in bioleaching: an overview. Miner Eng. 2019;136:140-54.
4. Xian Z, Xueduan L, Yili L, Fenliang F, Xiaoxia Z, Huaqun Y. Metabolic diversity and adaptive mechanisms of iron- and/or sulfur-oxidizing autotrophic acidophiles in extremely acidic environments. Env Microbiol Rep. 2016;8(5):738-51.

5. Temple KL, Colmer AR. The autotrophic oxidation of iron by a new bacterium: *Thiobacillus ferrooxidans*. J Bacteriol. 1951;62(5):605-11.
6. Falagán C, Johnson DB. *Acidithiobacillus ferriphilus* sp. nov., a facultatively anaerobic iron- and sulfur-metabolizing extreme acidophile. Int J Syst Evol Microbiol. 2016;66(1):206-11.
7. Hallberg KB, González-Toril E, Johnson DB. *Acidithiobacillus ferrivorans*, sp. nov.; facultatively anaerobic, psychrotolerant iron-, and sulfur-oxidizing acidophiles isolated from metal mine-impacted environments. Extremophiles. 2010;14(1):9-19.
8. Hedrich S, Johnson DB. *Acidithiobacillus ferridurans* sp. nov., an acidophilic iron-, sulfur- and hydrogen-metabolizing chemolithotrophic gammaproteobacterium. Int J Syst Evol Microbiol. 2013;63(11):4018-25.
9. Bryant RD, McGroarty KM, Costerton JW, Laishley EJ. Isolation and characterization of a new acidophilic *Thiobacillus* species (*T. albertis*). Can J Microbiol. 1983;29(9):1159-70.
10. Waksman SA, Joffe JS. Microorganisms concerned in the oxidation of sulfur in the soil: II. *Thiobacillus Thiooxidans*, a new sulfur-oxidizing organism isolated from the soil. J Bacteriol. 1922;7(2):239-56.
11. Hallberg KB, Lindström EB. Characterization of *Thiobacillus caldus* sp. nov., a moderately thermophilic acidophile. Microbiology. 1994;140(12):3451-6.
12. Kelly DP, Wood AP. Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov. and *Thermithiobacillus* gen. nov. Int J Syst Evol Microbiol. 2000;50(2):511-6.
13. Nitschke W, Bonnefoy V. Energy acquisition in low pH environments. In: Quatrini R, DB J, editors. *Acidophiles*: life in extremely acidic environments. Norfolk, UK: Caister Academic Press; 2016. p. 19-48.
14. Nuñez H, Moya-Beltrán A, Covarrubias PC, Issotta F, Cárdenas JP, González M, Atavales J, Acuña LG, Johnson DB, Quatrini R. Molecular systematics of the genus *Acidithiobacillus*: insights into the phylogenetic structure and diversification of the taxon. Front Microbiol. 2017;8:30.
15. Davis Jr. RA, Welty AT, Borrego J, Morales JA, Pendon JG, Ryan JG. Rio Tinto estuary (Spain): 5000 years of pollution. Environ Geol. 2000;39(10):1107-16.
16. González-Toril E, Llobet-Brossa E, Casamayor EO, Amann R, Amils R. Microbial ecology of an extreme acidic environment, the Tinto River. Appl Environ Microbiol. 2003;69(8):4853-65.
17. Rawlings DE. Heavy metal mining using microbes. Annu Rev Microbiol. 2002;56(1):65-91.
18. Rawlings DE. Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. Microb Cell Fact. 2005;4(1):13.
19. Sydow A, Krieg T, Mayer F, Schrader J, Holtmann D. Electroactive bacteria—molecular mechanisms and genetic tools. Appl Microbiol Biotechnol. 2014;98(20):8481-95.
20. Valdés J, Pedroso I, Quatrini R, Dodson RJ, Tettelin H, Blake R, Eisen JA, Holmes DS. *Acidithiobacillus ferrooxidans* metabolism: from genome sequence to industrial applications. BMC Genomics.

2008;9(1):597.

21. Lei Y, Shuang Z, Weidong W, Huixin H, Yanjie W, Gaobo Y, Peng C. Draft genome sequence of *Acidithiobacillus ferrooxidans* YQH-1. Genomics Data. 2015;6:269-70.
22. Peng C, Lei Y, Zhengrong W, Ruixiang X, Suyue L, Ningbo W, Ning L, Hongyu L. Draft genome sequence of extremely acidophilic bacterium *Acidithiobacillus ferrooxidans* DLC-5 isolated from acid mine drainage in Northeast China. Genomics Data. 2015;6:267-8.
23. Senol Cali D, Kim JS, Ghose S, Alkan C, Mutlu O. Nanopore sequencing technology and tools for genome assembly: computational analysis of the current state, bottlenecks and future directions. Briefings in Bioinformatics. 2018;20(4):1542-59.
24. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res. 2017;27(5):722-36.
25. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PloS One. 2014;9(11):e112963.
26. Hyatt D, Gwo-Liang C, LoCascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010;11(1):119.
27. Lowe TM, Chan PP. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res. 2016;44:54-7.
28. Lagesen K, Hallin P, Rødland EA, Stærfeldt H-H, Rognes T, Ussery DW. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 2007;35(9):3100-8.
29. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST server: rapid annotations using subsystems technology. BMC Genomics. 2008;9:75.
30. Kanehisa M, Goto S, Sato Y, Kawashima M, Furumichi M, Tanabe M. Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Res. 2013;42:199-205.
31. Galperin MY, Makarova KS, Wolf YI, Koonin EV. Expanded microbial genome coverage and improved protein family annotation in the COG database. Nucleic Acids Res. 2014;43:261-9.
32. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene Ontology: tool for the unification of biology. Nat Genet. 2000;25:25-9.
33. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19(9):1639-45.
34. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28(10):2731-9.
35. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;25(24):4876-82.

36. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406-25.
37. Tatusov RL, Fedorova ND, Jackson JD, Jacobs AR, Kiryutin B, Koonin EV, et al. The COG database: an updated version includes eukaryotes. BMC Bioinformatics. 2003;4:41.
38. Xian Z, Xueduan L, Liangzhi L, Guanyun W, Danli Z, Yili L, Bo M. Phylogeny, divergent evolution, and speciation of sulfur-oxidizing *Acidithiobacillus* populations. BMC Genomics. 2019;20(1):438.
39. Jensen AB, Webb C. Ferrous sulphate oxidation using *thiobacillus ferrooxidans*: a review. Process Biochem. 1995;30(3):225-36.
40. Wenbin N, Dejuan Z, Feifan L, Lei Y, Peng C, Xiaoxuan Y, Hongyu L. Quorum-sensing system in *Acidithiobacillus ferrooxidans* involved in its resistance to Cu^{2+}. Lett Appl Microbiol. 2011;53:84-91.
41. Jones P, Binns D, Hsin-Yu C, Fraser M, Weizhong L, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G. InterProScan 5: genome-scale protein function classification. Bioinformatics. 2014;30(9):1236-40.
42. Chengying J, Liu L, Xu G, Xiaoyan Y, Shuangjiang L, Poetsch A. Resolution of carbon metabolism and sulfur-oxidation pathways of *Metallosphaera cuprina* Ar-4 via comparative proteomics. J Proteomics. 2014;109:276-89.
43. Yue Z, Mengran Y, Shuang Z, Dan Z, Jiangong D, Weidong W, Lei Y. Iron and sulfur oxidation pathways of *Acidithiobacillus ferrooxidans*. World J Microb Biot. 2019;35(4):60.
44. Shuang Z, Lei Y, Weijia X, Peng C, Yu Z, Weidong W. *Acidithiobacillus ferrooxidans* and its potential application. Extremophiles. 2018;22(4):563-79.
45. Ruiqi Y, Guangxiu L, Tuo C, Wei Z, Gaosen Z, Sijing C. The complete genomic sequence of a novel cold-adapted bacterium, *Planococcus maritimus* Y42, isolated from crude oil-contaminated soil. Stand Genomic Sci. 2018;13:23.
46. Mochizuki S, Hiratsu K, Suwa M, Ishii T, Sugino F, Yamada K, Kinashi H. The large linear plasmid pSLA2-L of *Streptomyces rochei* has an unusually condensed gene organization for secondary metabolism. Mol Microbiol. 2003;48(6):1501-10.
47. Barrie Johnson D, Hallberg KB. Carbon, iron and sulfur metabolism in acidophilic micro-organisms. Adv Microb Physiol. 2008;54:201-55.
48. Ilbert M, Bonnefoy V. Insight into the evolution of the iron oxidation pathways. Biochim Biophys Acta. 2013;1827(2):161-75.
49. Yarzabal A, Appia-Ayme C, Ratouchniak J, Bonnefoy V. Regulation of the expression of the *Acidithiobacillus ferrooxidans rus* operon encoding two cytochromes c, a cytochrome oxidase and rusticyanin. Microbiology. 2004;150(7):2113-23.
50. Quatrini R, Jedlicki E, Holmes DS. Genomic insights into the iron uptake mechanisms of the biomining microorganism *Acidithiobacillus ferrooxidans*. J Ind Microbiol Biot. 2005;32(11-12):606-14.

51. Yarzábal A, Duquesne K, Bonnefoy V. Rusticyanin gene expression of *Acidithiobacillus ferrooxidans* ATCC 33020 in sulfur- and in ferrous iron media. Hydrometallurgy. 2003;71(1-2):107-14.
52. Brasseur G, Bruscella P, Bonnefoy V, Lemesle-Meunier D. The *bc1* complex of the iron-grown acidophilic chemolithotrophic bacterium *Acidithiobacillus ferrooxidans* functions in the reverse but not in the forward direction: Is there a second *bc1* complex? Biochim Biophys Acta. 2002;1555(1-3):37-43.
53. Quatrini R, Appia-Ayme C, Denis Y, Jedlicki E, Holmes DS, Bonnefoy V. Extending the models for iron and sulfur oxidation in the extreme acidophile *Acidithiobacillus ferrooxidans*. BMC Genomics. 2009;10:394.
54. Findlay AJ, Kamyshny A. Turnover rates of intermediate sulfur species (S_x^{2-}, S^0, $S_2O_3^{2-}$, $S_4O_6^{2-}$, SO_3^{2-}) in anoxic freshwater and sediments. Front Microbiol. 2017;8:2551.
55. Mangold S, Valdés J, Holmes D, Dopson M. Sulfur metabolism in the extreme acidophile *Acidithiobacillus caldus*. Front Microbiol. 2011;2:17.
56. Quatrini R, Appia-Ayme C, Denis Y, Ratouchniak J, Veloso F, Valdes J, et al. Insights into the iron and sulfur energetic metabolism of *Acidithiobacillus ferrooxidans* by microarray transcriptome profiling. Hydrometallurgy. 2006;83(1-4):263-72.

Tables

Table 1. Genome statistics of *Acidithiobacillus ferrooxidans* YNTRS-40.

Attribute	Value	% of total[a]
Genome size (bp)	3,257,037	100.00
DNA coding (bp)	2,940,490	90.28
DNA G + C (bp)	1,905,651	58.51
DNA scaffolds	2	100.00
Total genes	3419	100.00
Protein coding genes	3349	97.95
RNA genes	70	2.05
Pseudo genes	NA[b]	NA[b]
Genes in internal clusters	8	16.21
Genes with function prediction	1692	50.52
Genes assigned to COGs	1793	53.54
Genes with Pfam domains	2539	75.81
Genes with signal peptides	NA[b]	NA[b]
Genes with transmembrane helices	NA[b]	NA[b]
CRISPR repeats	0	0

[a]The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome; [b]NA, not applicable.

Table 2. Number of genes associated with general COGs functional categories.

Code	Value	% age	Description
J	172	5.14	Translation, ribosomal structure and biogenesis
A	0	0	RNA processing and modification
K	118	3.52	Transcription
L	126	3.76	Replication, recombination and repair
B	1	0.03	Chromatin structure and dynamics
D	29	0.87	Cell cycle control, Cell division, chromosome partitioning
Y	0	0	Nuclear structure
V	90	2.69	Defense mechanisms
T	87	2.60	Signal transduction mechanisms
M	162	4.84	Cell wall/membrane/envelope biogenesis
N	20	0.60	Cell motility
Z	0	0	Cytoskeleton
W	14	0.42	Extracellular structures
U	62	1.85	Intracellular trafficking, secretion, and vesicular transport
O	96	2.87	Posttranslational modification, protein turnover, chaperones
C	129	3.85	Energy production and conversion
G	93	2.78	Carbohydrate transport and metabolism
E	137	4.09	Amino acid transport and metabolism
F	53	1.58	Nucleotide transport and metabolism
H	108	3.22	Coenzyme transport and metabolism
I	72	2.15	Lipid transport and metabolism
P	135	4.03	Inorganic ion transport and metabolism
Q	34	1.02	Secondary metabolites biosynthesis, transport and catabolism
R	138	4.12	General function prediction only
S	51	1.52	Function unknown
X	88	2.63	Mobilome: prophage, transposons
-	1334	39.83	Not in COGs

The total is based on the total number of protein coding genes (3349) in the genome.

Figures

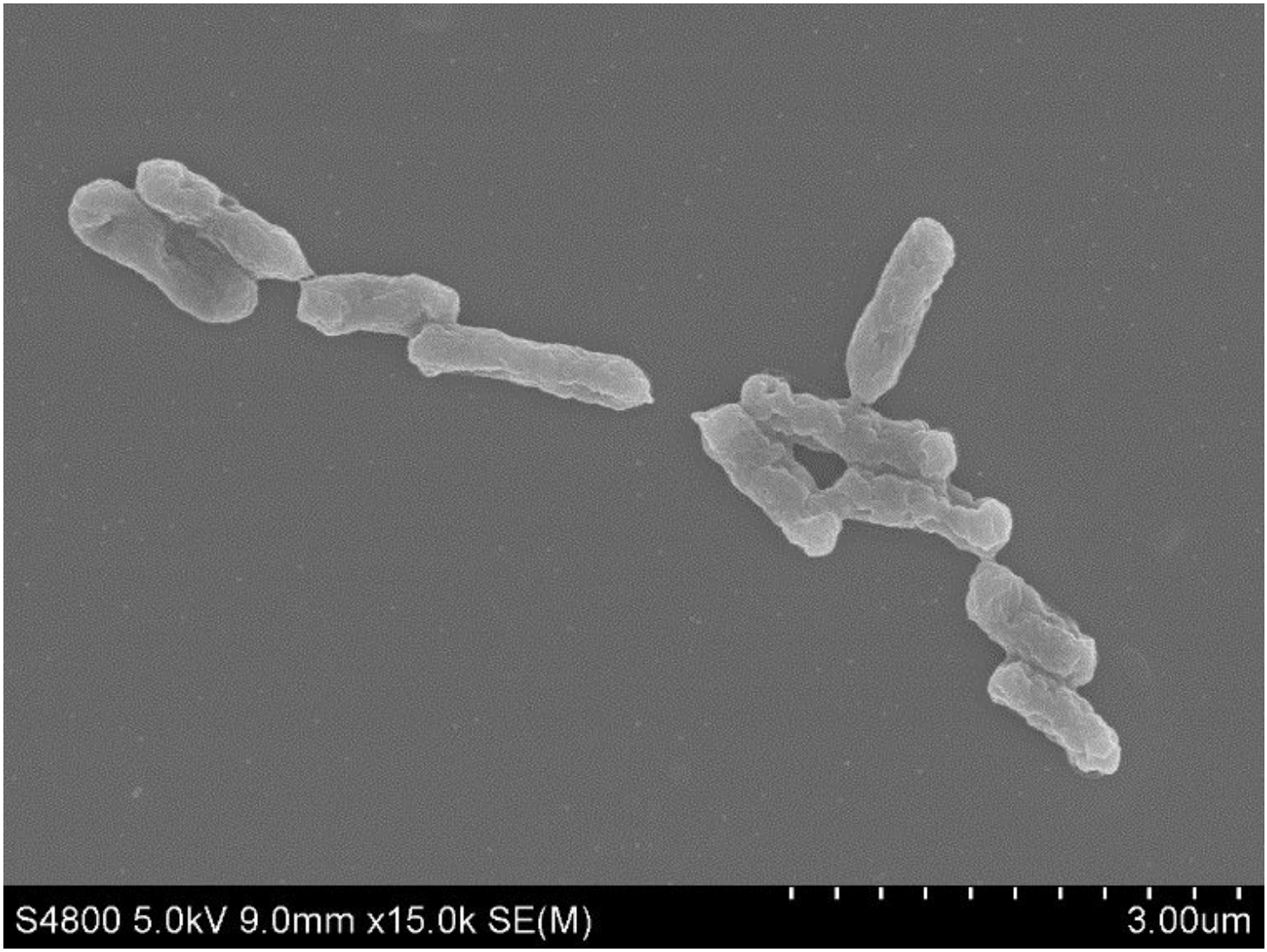

Figure 1

Scanning electron micrograph of Acidithiobacillus ferrooxidans YNTRS-40.

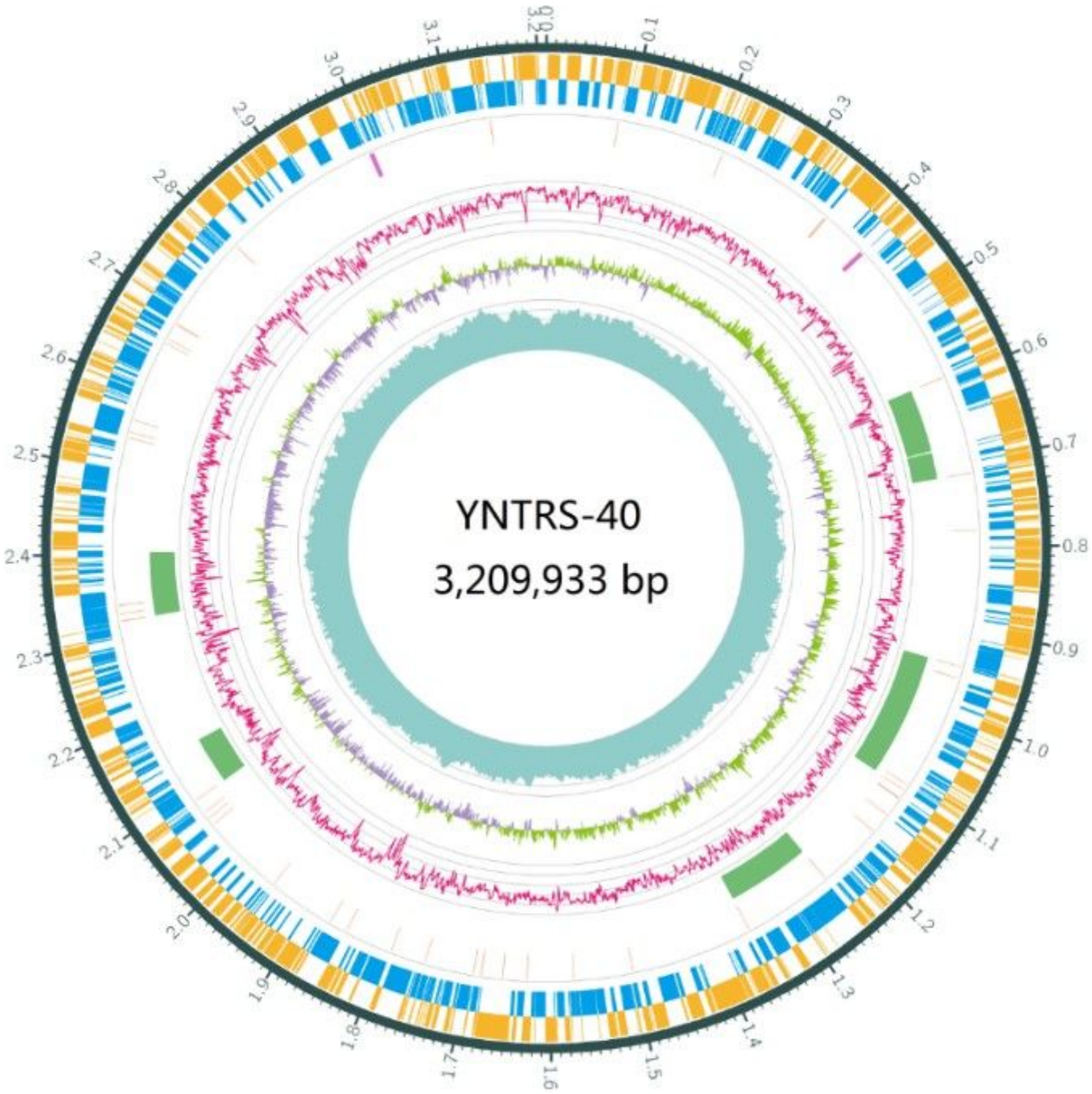

Figure 2

Circular chromosome genome map of Acidithiobacillus ferrooxidans YNTRS-40. (From the outside to the center, genes on direct strand, genes on complementary strand, tRNAs (orange), rRNAs (purple), CRISPR (blue) and genomic island (green), GC-skew, sequencing depth are displayed)

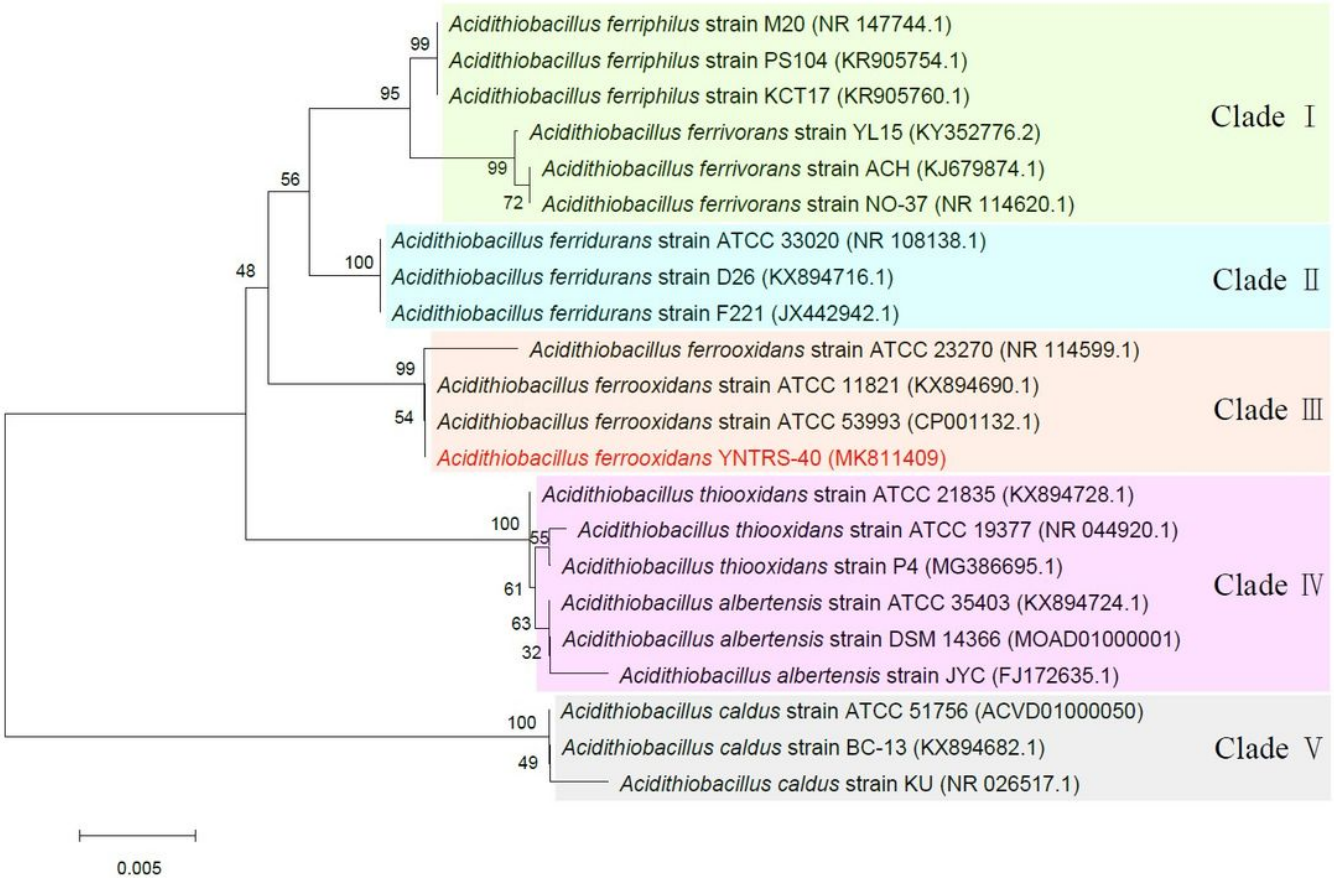

Figure 3

Phylogenetic tree based on 16S rRNA gene sequence of Acidithiobacillus ferrooxidans YNTRS-40 and its relatives. (Bootstrap values were calculated by MEGA5.05 using the neighbor joining method from 1000 replications. Bar, 0.005 nucleotide substitutions per nucleotide position)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryinformation.docx