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Complete genome sequence of *Erythrobacter gangjinensis* CGMCC 1.15024^T with two chromosomes

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ABSTRACT

Erythrobacter gangjinensis CGMCC 1.15024^T (JCM 15420^T = KCTC 22330^T) was isolated from seawater of a shellfish farm in Gangjin Bay, Korea. In this study, the genome of *E. gangjinensis* CGMCC 1.15024^T was sequenced and comprised of two circular chromosomes, named chromosome I and II, with the size of 2,495,029 and 229,922 bp, respectively. The two chromosomes have similar DNA G + C content (62.7 and 62.9%) and percentages of coding regions (91.2 and 89.8%). The comparison of two annotated chromosomes revealed that either chromosome harbored unique genes (e.g. fatty acid biosynthesis genes in chromosome I and catalase-encoding gene in chromosome II). *E. gangjinensis* CGMCC 1.15024^T is the first strain with two chromosomes reported in genus *Erythrobacter* and provides new insights into understanding of the evolution and phenotypes of genus *Erythrobacter*.

1. Introduction

The presence of two unique circular chromosomes in a bacterium was first reported in *Rhodobacter sphaeroides* van Niel's ATH 2.4.1^T (Suwanto and Kaplan, 1989). Subsequently, bacteria with multiple chromosomes were found in more bacterial taxa, such as *Agrobacterium* (Slater et al., 2009), *Cupriavidus* (Janssen et al., 2010), *Pseudoalteromonas* (Rong et al., 2016) and *Vibrio* (Yamaichi et al., 2007). Bacteria with multiple chromosomes usually have two circular chromosomes with the larger chromosome as the primary chromosome and the smaller one as the accessory chromosome (Choudhary et al., 2012). Previous studies revealed that the primary chromosome harbored more essential genes for cellular growth and the accessory chromosome encoded more flexible proteins for bacteria to develop their metabolic diversity (Cooper et al., 2010; Rong et al., 2016). The studies on the bacteria with multiple chromosomes provide new insights into evolution and cellular mechanisms (Choudhary et al., 2012).

Erythrobacter, belonging to the order *Sphingomonadales* of *Alphaproteobacteria*, is Gram-negative (Tonon et al., 2014). *Erythrobacter* strains are chemoorganotrophic and non-spore-forming (Tonon et al., 2014). They are widely distributed in the marine environments (Tonon et al., 2014). Although three complete *Erythrobacter* genomes, including *E. atlanticus* s21-N3^T (DDBJ/EMBL/GenBank accession number: CP011310 and CP015441) isolated from southern Atlantic Ocean, *E. litoralis* HTCC2594 (DDBJ/EMBL/GenBank accession number: CP000157) isolated from Sargasso Sea and *Erythrobacter* sp. NAP1 (DDBJ/EMBL/GenBank accession number: NZ_AAMW00000000) isolated from northwestern Atlantic Ocean, have been reported, none of them contains multiple chromosomes (Oh et al., 2009; Koblížek et al., 2011; Zhuang et al., 2015). Here we sequenced the genome of *Erythrobacter gangjinensis* CGMCC 1.15024^T, which was isolated from seawater of a shellfish farm in Gangjin Bay, Korea (Lee et al., 2010). The sequencing reads were assembled into two circular chromosomes. As first reported in genus *Erythrobacter*, the two chromosomes in *E. gangjinensis* CGMCC 1.15024^T would broaden our understandings of evolutionary history and phenotypic characteristics of genus *Erythrobacter*.

2. Data description

E. gangjinensis CGMCC 1.15024^T was obtained from the China General Microbiological Culture Collection Center. The cells of *E. gangjinensis* CGMCC 1.15024T were cultivated in Marine Broth 2216 (BD Difco™, Sparks, USA), pH 7.5 at 30 °C for 48 h. The cells were centrifuged at 12,000 ×*g* for 30 s at room temperature. The genomic DNAs were

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extracted using the AxyPre™ Bacterial Genomic DNA Miniprep Kit (Corning Life Sciences, Suzhou, China). The genome sequencing of *E. gangjinensis* CGMCC 1.15024^T was performed using Solexa paired-end sequencing technology (HiSeq2000 system, Illumina, USA) with a whole-genome shotgun technology in Novogene Corporation (Beijing). One paired-end library with insert length of 500 bp was constructed. A total of 890,721,750 bp clean data were generated, representing approximately 327 fold genomic coverage. De novo assembly was performed using ABySS 1.9.0 (Simpson et al., 2009). The assembled draft genome was constituted of two contigs and completed by PCR reactions with PrimeSTAR® GXL DNA polymerase (TaKaRa, Dalian, China) and Sanger sequencing using an Applied Biosystems™ PRISM 3130XL DNA analyzer (Thermo Fisher Scientific, Foster City, USA).

The tRNA genes were found by tRNAscan-SE v1.3 (Lowe and Eddy, 1997) and the rRNA genes were identified through RNAmmer 1.2 server (Lagesen et al., 2007). The open reading frames (ORFs) were predicted and annotated by Glimmer v.3.0 (Delcher et al., 2007) and Rapid Annotation using Subsystem Technology (RAST) server online (Overbeek et al., 2014). The predicted genes were analyzed by using Clusters of Orthologous Groups (COG) database (Tatusov et al., 2001) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Aoki and Kanehisa, 2005). Coding sequences (CDSs) with signal peptides and trans-membrane helices were identified through SignalP 4.1 Server (Petersen et al., 2011) and TMHMM Server v.2.0 (Krogh et al., 2001), respectively. The translated ORFs of two chromosomes were compared by using OrthoMCL v2.0.9 (Li et al., 2003; Fischer et al., 2011) to identify orthologous clusters (OCs). The origins of chromosome replication (*oriC*) was predicted by using Ori-Finder (Gao and Zhang, 2008). Circular figures of *E. gangjinensis* CGMCC 1.15024^T were visualized by CGView (Stothard and Wishart, 2005).

Table 1
General features of *Erythrobacter gangjinensis* CGMCC 1.15024^T and MIGS mandatory information.

Items	Description
General features	
Gram stain	Negative
Cell shape	Rod
Motility	Non-motile
Colour of colonies	Orange
Temperature	15 to 37 °C
Sea salt concentration	1 to 7%
pH range	6.0 to 10.0
MIGS data	
Investigation type	Bacteria_archaea
Project_name	Genome sequence of *Erythrobacter gangjinensis*
Lat_Lon	34°27′ N, 126°47′ E
Depth	Not reported
Geo_loc_name	Gangjin Bay, Korea
Coll_date	Not reported
Env_biome	Bay, [ENVO:00000032]
Env_feature	Aquacultural site, [ENVO:00000294]
Env_material	Water, [ENVO:00002006]
Env_package	Sea water, [ENVO:00002149]
Num_replicons	2
Estimated_size	2,724,951 bp
Source_mat_id	CGMCC 1.15024^T = K7-2^T = JCM 15420^T = KCTC 22330^T
Ref_biomaterial	PMID: 19671726
Trophic_level	Chemoorganotroph
Rel_to_oxygen	Aerobic
Isol_growth_condt	PMID: 19671726
Lib_reads_seqd	7,125,774 reads
Seq_meth	ABI 3130XL, Illumina HiSeq 2000
Assembly data	
Assembly method	ABySS 1.9.0
Assembly name	Egan_v1.0
Genome coverage	~327×
Finishing strategy	Prime design, PCR and Sanger sequencing

The genomic features of *E. gangjinensis* CGMCC 1.15024^T are shown in Tables 1 and 2. The genome of *E. gangjinensis* CGMCC 1.15024^T contains two circular replicons with a total size of 2,724,951 bp. To identify whether the replicons are plasmid or accessory chromosome, the elements involved in replication were analyzed in both *E. gangjinensis* CGMCC 1.15024^T and its relative *E. atlanticus* s21-N3^T, which harbors a plasmid with a size of 213,959 bp, since the replications of chromosome and plasmid are different. Chromosome replication initiates at *oriC* when ATP binds DnaA (Gao and Zhang, 2008), while the initiation of plasmid replication needs plasmid replication protein RepA (del Solar et al., 1998). The chromosomal replication initiator protein DnaA are present in both genetic replicons of *E. gangjinensis* CGMCC 1.15024^T (locus_tag as BMF35_a0001 and BMF35_b0001) and the chromosome of *E. atlanticus* s21-N3^T (CP97_04145), while plasmid replication protein RepA (CP97_14866) is only found in the plasmid of *E. atlanticus* s21-N3^T. Besides, *oriC*s can be found in both circular replicons of *E. gangjinensis* CGMCC 1.15204^T and the chromosome of *E. atlanticus* s21-N3^T, rather than in the plasmid of *E. atlanticus* s21-N3^T (Supplementary Table S1). These results suggest that both of the two circular replicons of *E. gangjinensis* CGMCC 1.15204^T are chromosomes.

The two chromosomes have similar characteristics, such as DNA G + C content (62.7 and 62.9%) and percentages of coding regions (91.2 and 89.8%), CDSs assigned to COG (73.5 and 73.1%) and KEGG (45.2 and 45.3%) and CDSs with signal peptides (14.5 and 11.4%). However, the operon of 16S–23S-5S rRNA gene only exists in the larger chromosome (chromosome I). Moreover, chromosome I has 40 tRNA genes, while the smaller chromosome (chromosome II) harbors 3 tRNA genes. Besides, chromosome II has higher percentage of CDSs (43.1%) with transmembrane helices than chromosome I (24.5%).

A total of 2598 OCs (2420 OCs in chromosome I and 227 OCs in chromosome II) were identified, among which 49 OCs were common between chromosome I and chromosome II, while 2371 unique OCs in chromosome I and 178 unique OCs in chromosome II (Fig. 1a and b). Besides, predicted KEGG pathways indicated that more pathways were related to chromosome I (201) than chromosome II (102). Some genes in chromosome I encoded proteins playing an important role in basic metabolic pathways, such as glycolysis and TCA cycle (Supplementary Table S1). Furthermore, the comparison of KEGG pathways between two chromosomes showed that genes involved in some basic metabolic pathways were only in chromosome I, such as those in fatty acid biosynthesis (Supplementary Table S2). In addition, genes in chromosome I mostly encoded proteins assigned to amino acid transport and metabolism (8.4%; 170/2014), translation, ribosomal structure and biogenesis (7.5%; 151/2014) and cell wall/membrane/envelope biogenesis (7.9%; 159/2014) in COG categories (Fig. 1c). Hence, chromosome I in *E. gangjinensis* CGMCC 1.15024^T might support cellular growth basically with a large number of genes concerning DNA replication and basic metabolic pathways, including DNA gyrase-encoding genes *gyrA* and *gyrB*, phosphofructokinase 1-encoding gene *pfkA*, pyruvate kinase *pyk*, etc.

Table 2
Comparative general genomic features of chromosome I and II within *Erythrobacter gangjinensis* CGMCC 1.15024^T.

Features	Chromosome I	Chromosome II
Size (bp)	2,495,029	229,922
DNA G + C content (%)	62.7	62.9
CDSs	2426	227
CDSs assigned to COGs (percentages)	1783 (73.5%)	166 (73.1%)
CDSs assigned to KEGG (percentages)	1097 (45.2%)	103 (45.3%)
CDSs with signal peptides (percentages)	352 (14.5%)	26 (11.4%)
CDSs with trans-membrane helices (percentages)	595 (24.5%)	66 (43.1%)
rRNA operon (16S-23S-5S rRNA)	1	0
tRNAs	40	3

Although more OCs and pathways were identified in chromosome I, some genes functioning in basic metabolic pathways were only in chromosome II, such as glucokinase-encoding gene *glk* (BMF35_b0136) in glycolysis and aconitate hydratase-encoding gene *acnA* (BMF35_b0035) in TCA cycle. OCs annotated with identified functions in chromosome II were mostly related to inorganic ion transport and metabolism (9.3%; 17/182 in COG categories (Fig. 1c). For example, catalase activity preventing damages to cells from hydrogen peroxide was identified as positive in *E. gangjinensis* CGMCC 1.15024^T (Lee et al., 2010), and the gene encoding catalase (BMF35_b0023) was only located in chromosome II. Moreover, CDSs in chromosome II with trans-membrane helices played important roles in transporting substances through membrane. For example, genes encoding magnesium and cobalt transport protein (BMF35_b0046), molybdenum transport system permease protein (BMF35_b0091), potassium channel protein (BMF35_b0149) were only in chromosome II.

Some *Erythrobacter* strains are aerobic anoxygenic phototrophic bacteria which contain bacteriochlorophyll *a* (Tonon et al., 2014), but bacteriochlorophyll *a* is not present in *E. gangjinensis* CGMCC 1.15204^T (Lee et al., 2010), and genes involved in bacteriochlorophyll *a* biosynthesis (*bchC*, *bchF*, *bchG*, *bchH*, *bchJ*, *bchM*, *bchP* and *bchX*) are not annotated in the genome of *E. gangjinensis* CGMCC 1.15204^T. So far, the genome size of *E. gangjinensis* CGMCC 1.15204^T (2.71 Mbp) is the smallest among all of complete *Erythrobacter* genomes (*E. atlanticus* s21-N3^T: 3.22 Mbp, *E. litoralis* HTCC2594: 3.05 Mbp and *Erythrobacter* sp. NAP1: 3.19 Mbp). The genomic comparison of these four complete *Erythrobacter* genomes reveals that *E. gangjinensis* CGMCC 1.15204^T has 17 unique OCs (β-glucuronidase, polygalacturonase, polysaccharide deacetylase, etc.), and lacks 132 OCs (multicopper oxidase, nickel-cobalt-cadmium resistance protein, plasmid conjugative transfer protein, etc.) shared by other three *Erythrobacter* strains (Supplementary Fig. S1).

The complete genome of *Erythrobacter gangjinensis* CGMCC 1.15024^T contains two circular chromosomes. The finding of *Erythrobacter* with two chromosomes broadens our knowledge in the genomic and phenotypic features of genus *Erythrobacter*, and provides insights into understanding of the evolution within genus *Erythrobacter*.

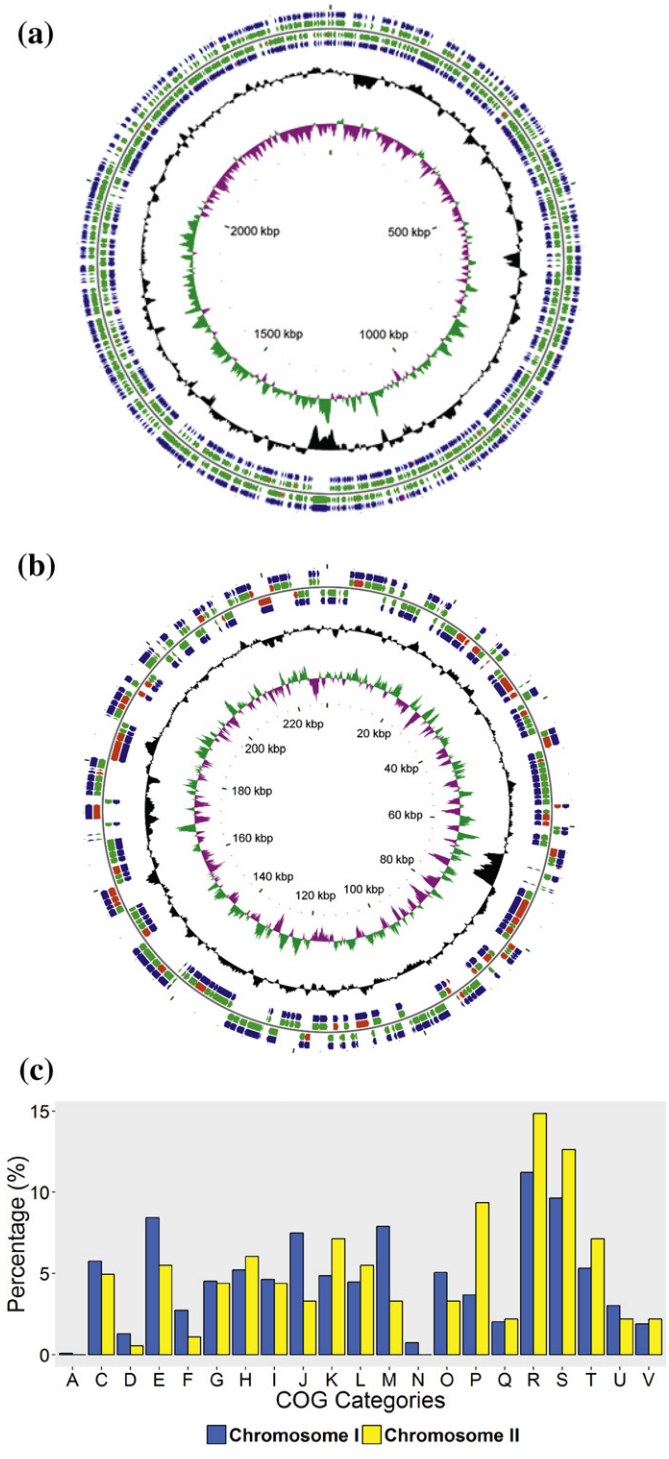

Fig. 1. Circular representations of chromosome I (a) and II (b) in the genome of *Erythrobacter gangjinensis* CGMCC 1.15024^T genome. From the outside to the center: CDSs, rRNA genes and tRNA genes on forward strand, shared and unique OCs on forward strand, shared and unique OCs on reverse strand, CDSs, rRNA genes and tRNA genes on reverse strand, DNA G + C content and GC skew. CDSs, rRNA genes, tRNA genes, shared OCs and unique OCs are illustrated in different colours: blue indicates CDSs; brown indicates tRNA genes; purple indicates rRNA genes; red indicates shared OCs; and green indicates unique OCs. Distribution of COG categories within two chromosomes of *Erythrobacter gangjinensis* CGMCC 1.15024^T (c). A: RNA processing and modification; C: energy production and conversion; D: cell cycle control, mitosis and meiosis; E: amino acid transport and metabolism; F: nucleotide transport and metabolism; G: carbohydrate transport and metabolism; H: coenzyme transport and metabolism; I: lipid transport and metabolism; J: translation, ribosomal structure and biogenesis; K: transcription; L: replication, recombination and repair; M: cell wall/membrane/envelope biogenesis; N: cell motility; O: post translational modification, protein turnover and chaperones; P: inorganic ion transport and metabolism; Q: secondary metabolites biosynthesis, transport and catabolism; R: general function prediction only; S: function unknown; T: signal transduction mechanisms; U: intracellular trafficking and secretion; V: defense mechanisms.

3. Nucleotide sequence accession number

The complete genome sequence of *E. gangjinensis* CGMCC 1.15024^T was deposited in DDBJ/EMBL/GenBank under the accession number CP018097 for chromosome I and CP018098 for chromosome II.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.margen.2017.02.004.

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