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Determination of Phenotypic and Genome Characteristics of *Chryseobacterium* sp. C-204 Strain Isolated from Rainbow Trout

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Summary: In recent years, species in the *Chryseobacterium* genus have emerged as opportunistic fish pathogens that can cause death in fish in many countries. In the last decade, *C. aahli, C. oncorhynchi, C. chaponense,* and *C. piscicola* have been reported to cause systemic infections in fish. In the present study, *Chryseobacterium* sp. C-204 was isolated from 1g weight rainbow trout showing clinical signs such as abnormal swimming, dorsal skin ulceration, darkening in color, and bilateral exophthalmos. The detailed phenotypic characteristics of the C-204 were characterized by API 20NE, and the BIOLOG GEN III system includes 106 phenotypes. Antimicrobial susceptibility of the C-204 was also determined by the broth microdilution method against five antimicrobial agents commonly used in the Aquaculture. Sequence-based identification was done using 16S rRNA genome sequencing. The genome structure of the C-204 was revealed by using next-generation genome sequencing with reading a total of 24195304 bases and assembled in 4012452 base. Genome-based species delineation of C-204 was done 100 different housekeeping gene regions and 50 the closest *Chryseobacterium* species with Automated Multi-Locus Species Tree (autoMLST, https://automlst.ziemertlab.com). Antimicrobial resistance genes (AMR) and virulence genes in the C-204 genome were identified using the Virulence Factor Database (VFDB) NCBI-reference antimicrobial resistance genes database. The 16S rRNA sequence of C-204 isolate had similarities with the *C. aquaticum* (99.65%) and *C. greenlandense* (98.95%) in GenBank. In parallel 19 biochemical tests, C-204 isolate can be differentiated from the closest type strains by nitrate reduction and inability to produce acid from glucose. With regard to antimicrobial susceptibility, the C-204 isolate can grow even at high antimicrobial concentrations determined for *Flavobacteriaceae*. According to genome-based species delineation, the C-204 isolate was identified as *Chryseobacterium aquaticum* subsp *greenlandense.* 13 virulence and eight AMR genes were detected in the genome of the C-204 isolate. Conclusively, the detailed phenotypic characteristic includes 106 biochemical test and genome structure of C-204 isolate by whole genome sequencing were determined.
Key words: Antimicrobial resistance genes, *Chryseobacterium sp.*, virulence genes

Gökkuşağı Alabalıklarından Izole Edilen *Chryseobacterium* sp. C-204 Suşunun Fenotipik ve Genom Özelliklerinin Belirlenmesi

Özet: Son yıllarda *Chryseobacterium* cinsinde bulunan türler, birçok ülkede balıklarda ölümlere neden olabilen fırsatçı balık patojenleri olarak ortaya çıkmaktadır. Yalnızca son on yılda *C. aahli, C. oncorhynchi, C. chaponense ve C. piscicola*'nın balıklarda sistemik enfeksiyonlara neden olduğu bildirilmiştir. Bu çalışmada, *Chryseobacterium* sp. C-204, anormal yüzme, sırt lezyonu, renkte koyulaşma ve iki taraflı egzoftalmi gibi klinik belirtiler gösteren 1 gram ağırlığındaki gökkuşağı alabalığından izole edildi. C-204'ün 106 farklı testi içeren fenotipik özellikleri API 20NE ve BIOLOG GEN III sistemi ile karakterize edildi. C-204'ün antimikrobiyal duyarlılığı, su ürünleri yetiştiriciliğinde yaygın olarak kullanılan beş farklı antimikrobiyal ajana karşı broth mikrodilüsyon yöntemiyle belirlendi. Dizi analizi bazlı tür tanımlama, 27F ve 1387R primerleri kullanılarak yapıldı. C-204'ün tüm genom analizinde, yeni nesil dizilime sistemi kullanıldı ve toplam 24195304 okuma elde edildi. Bu okumalar birleştirilerek 4012452 baz uzunluğunda taslak genom elde edildi. C-204'ün genoma dayalı tür tanımlaması, 100 farklı korunmuş gen bölgesi ve en yakın 50 *Chryseobacterium* türü ile autoMLST sisteminde (https://automlst.ziemertlab.com) yapıldı. C-204 genomundaki antimikrobiyal direnç genleri (AMR) ve virülans genleri, NCBI referans antimikrobiyal direnç genleri veritabanı ve Virülans Faktör Veritabanı (VFDB) kullanılarak tanımlandı. C-204 izolatının 16S rRNA gen bölgesinin, GenBankta *C. aquaticum* ile %99.65 ve *C. greenlandense* ile %98.95 oranında benzerliklere sahip olduğu belirlenmiştir. Ortak yapılmış olan 19 biyokimyasal testte, C-204 izolatı nitrat indirgeyebilmesi ve glikozdan asit üretememesi testleri ile diğer tip suşlardan ayrılabildiği belirlenmiştir. Antimikrobiyal duyarlılıkla ilgili olarak, C-204 izolatının yüksek antimikrobiyal konsantrasyonlarında bile üreyebildiği tespit edilmiştir. Genom bazlı tür tanımlamasına göre, C-204 izolatı, *Chryseobacterium aquaticum* subsp *greenlandense* olarak tanımlandı. Ayrıca, C-204 suşunun genomunda 13 virülans ve sekiz AMR geni tespit edildi. Çalışmamızda sonuç olarak, C-204 izolatının 106 biyokimyasal özellik içeren detaylı fenotipik ve tüm genom dizi analizine dayalı genom yapısı belirlenmiştir.
Anahtar kelimeler: Antimikrobiyal direnç geni, *Chryseobacterium* sp., virülens geni

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Introduction

In the last thirty years, aquaculture has grown rapidly in Turkey, and the total amount of products obtained from 2286 farms in 2018 reached 276502 tons, and total fisheries exports reached $952 million (BSGM, 2020). With the rapid development of aquaculture, economic losses from diseases caused by pathogens have also increased. The family of *Flavobacteriaceae* has a considerable sizeable ecological habitat. The species in this family; Invertebrates, amphibians, reptiles, birds, and even human beings can cause infections in mammals. Among the species causing fish infection in the *Flavobacteriaceae* family, there are significant species such as *Flavobacterium* sp., *Tenacibaculum* sp., and *Chryseobacterium* sp. It has been reported that *Chryseobacterium* species are isolated from different clinical cases (pneumonia, peritonitis, surgical wound infections, burn wounds, eye infections, newborn pneumonia, etc.) in humans (Loch and Faisal, 2015a). *Chryseobacterium* species have been reported to cause economic losses in many fish species on different continents. Only in recent years *C. piscicola*, *C. chaponense*, *C. aahli,* and *C. oncorhynchi* have been reported to cause systemic infections in fish (Hugo et al., 2019).

There are not many studies on whether the detected *Chryseobacterium* species are the main cause of the disease. In a limited number of studies, it was reported that *C. balustinum, C. piscicola,* and *C. aahli* fulfill Koch postulates, but there is no study on other *Chryseobacterium* species (Ilardi et al., 2009; Loch and Faisal, 2015b, 2014).

In our study, it was aimed to determine the comprehensive phenotypic characterization, genome analysis, and antimicrobial susceptibility of *C. aquaticum* subsp. *greenlandense* C-204 isolate isolated from rainbow trout showing disease symptoms such as dorsal skin ulceration, darkening in color, and bilateral exophthalmia.

Materials and methods

Bacterial isolate *and phenotypic characterization*

In our study, the C-204 isolate was isolated from a rainbow trout, weighing about 1 gram in the trout farm located in the Aegean region, 2015, showing signs of abnormal swimming, dorsal lesion, darkening in color, and bilateral exophthalmos was used. This research was approved by Bursa Uludag University, the Local Ethics Commission (report 2012-14/04).

In primary isolation, Tryptone Yeast Extract Salts (TYES) agar was used, and the agent was incubated at 25°C for 48 hours. Conventional microbiological tests such as Gram staining, motility, oxidase, presence or absence of flexirubin pigment, catalase activity were used in phenotypic characterization of strains (Loch et al., 2013). The morphological and biochemical profiles of C-204 isolate were determined using the Biolog GEN III microplate (Biolog, Hayward, CA, USA) and API 20NE (Biomerieux, France) tests. Unlike the manufacturer's protocol, the incubation temperature and time were modified to optimum growth values of C-204 isolate (48h incubation at 25°C).

Sequence analysis based on 16S rRNA gene

DNA extraction of C-204 isolate was performed according to the spin column filtration (QIAamp DNA Minikit; Qiagen, Hilden, Germany) method. PCR and sequence analysis was performed using 27F (5'-AGA GTT TGA TCM TGG CTC 118 AG-3') and 1387R (5'-GGG CGG WGT GTA CAA GGC-3') based on the 16S rRNA gene region (Loch et al., 2013). The obtained chromatograms were aligned and identified in the BLAST (Basic Local Alignment Search Tool) server database in the National Center for Biotechnology Information (NCBI).

Whole-genome sequencing

The sequencing library was prepared using Nextera XT DNA Library Preparation Kit, and sequencing was done by Illumina NovaSeq 6000 platform as paired-end (PE) 2x150 bases reads with using a 500-cycle MiSeq reagent kit (Cooper et al., 2015). The Fastq file obtained from the sequence analysis was uploaded to Geneious Prime (version 2020.1.2). The reads with low-quality scores (<20) or poly-Ns and adaptor contamination were then trimmed using BBDuk as implemented in Geneious Prime (Kearse et al., 2012). The high-quality reads of C-204 were assembled into costig by de novo assembly using SPAdes assembler 3.13.0 (Bankevich et al., 2012). The draft genome sequence has been recorded to Genbank with SAMN15009847 and PRJNA634826 BioSample and BioProject number, respectively.

Genomic data analysis

Genome-based species delineation of C-204 was created using Automated Multi-Locus Species Tree (autoMLST, https://automlst.ziemertlab.com). In phylogenetic analysis, 100 different housekeeping gene regions of C-204 and 50 the closest *Chryseobacterium* species genomes were used. A high-resolution species tree was created using the web-based autoMLST program (Alanjary et al., 2019). Annotation of the C-204 genome was performed using NCBI-Automatic Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al., 2016). Virulence genes in the C-204 genome were identified using the Virulence Factor Database (VFDB), while the antimicrobial resistance genes were identified by searching BLASTX (E-value <1e-50) in the Geneious Prime software using the NCBI-reference antimicrobial resistance genes database (Feldgarden et al., 2019; Kearse et al., 2012; Lee et al., 2019).

Table 1. All phenotypic characteristics of the *C. aquaticum* KCTC 12483, *C. greenlandense* UMB34, and *Chryseobacterium sp*. C-204 isolate

	Dextrin	**D-Maltose**	**D-Trehalose**	**D-Cellobiose**	**Gentiobiose**	**Sucrose**	**D-Turanose**	**Stachyose**	**+ Control**	**pH 6**	**pH 5**
	C(+), A(+)	C(+), A(+), G(+)	C(+), A(+), G(+)	C(-), A(+)	C(+), A(+), G(+)	C(+), A(+), G(+)	C(-), A(+)	C(-)		C(+)	C(-)
D-Raffinose	**α-D-Lactose**	**D-Melibiose**	**β-Methyl-DGluco-side**	**D-Salicin**	**N-Acetyl-D-Glucosamine**	**N-Acetyl-β-DMannosa-mine**	**N-Acetyl-DGalactosa-mine**	**N-Acetyl Neuraminic Acid**	**1% NaCl**	**4% NaCl**	**8% NaCl**
C(-), A(+)	C(-), A(-)	C(-), A(+)	C(-), A(-)	C(-), A(-)	C(-), A(-), G(-)	C(-), A(-), G (-)	C(-), A(+)	C(-)	C(+)	C(-)	C(-)
α-D-Glucose	**D-Mannose**	**D-Fructose**	**D-Galac-tose**	**3-Methyl Glucose**	**D-Fucose**	**L-Fucose**	**L-Rhamnose**	**Inosine**	**1% Sodium Lactate**	**Fusidic Acid**	**D-Serine**
C(+), A(+), G(+)	C(+), A(+), G(-)	C(+), A(+), G(+)	C(-), A(+)	C(-)	C(-), A(-)	C(-), A(-)	C(+), A(+)	C(-), A(-)	C(+)	C(-)	C(-), A(-)
D-Sorbitol	**D-Mannitol**	**D-Arabitol**	**myo-Inositol**	**Glycerol**	**D-Glucose 6-PO4**	**D-Fructose6-PO4**	**D-Aspartic Acid**	**D-Serine**	**Trolean-domycin**	**Ri-famycin SV**	**Minocy-cline**
C(-), A(+)	C(-), A(-), G (-)	C(-), A(+)	C(-), A(+)	C(-), A(-)	C(+), A(+)	C(+)	C(-)	C(-), A(-)	C(+)	C(-)	C(-)
Gelatin	**Glycyl-L-Proline**	**L-Alanine**	**L-Arginine**	**L-Aspartic Acid**	**L-Glutamic Acid**	**L-Histidine**	**L-Pyroglutamic Acid**	**L-Serine**	**Lincomycin**	**Guani-dine HCl**	**Niaproof 4**
C(+), A(+), G(+)	C(+)	C(-), A(+)	C(-), A(-), G(-)	C(+), A(+)	C(+), A(+)	C(-), A(-)	C(-), A(+)	C(-), A(+)	C(-)	C(+)	C(-)

Pectin	**D-Galacturonic Acid**	**L-Galactonic Acid Lactone**	**D-Gluconic Acid**	**D-Glucuronic Acid**	**Glucu-ronamide**	**Mucic Acid**	**Quinic Acid**	**D-Saccharic Acid**	**Vancomycin**	**Tetrazolium Violet**	**Tetrazolium Blue**
C(+)	C(+), A(+)	C(+), A(-)	C(-), A(+)	C(-), A(-)	C(-), A(-)	C(-)	C(-)	C(-), A(-)	C(-)	C(+)	C(+)
p-Hydroxy Phenylacetic Acid	**Methyl Py-ruvate**	**D-Lactic Acid Methyl Ester**	**L-Lactic Acid**	**Citric Acid**	**α-Keto-Glutaric Acid**	**D-Malic Acid**	**L-Malic Acid**	**Bromo-Succinic Acid**	**Nalidixic Acid**	**Lithium Chloride**	**Potassium Tellurite**
C(-), A(-)	C(+), A(+)	C(-)	C(+), A(-)	C(-), A(-)	C(-), A(-)	C(-)	C(-)	C(-), A(-)	C(+)	C(-)	C(+)
Tween 40	**γ-Amino-Butryric Acid**	**α-Hydroxy Butyric Acid**	**β-Hydroxy-D, LButyric Acid**	**α-Keto-Butyric Acid**	**Acetoacetic Acid**	**Propionic Acid**	**Acetic Acid**	**Formic Acid**	**Aztreonam**	**Sodium Butyrate**	**Sodium Bromate**
C(+), A(+)	C(-), A(-)	C(-), A(-)	C(-), A(-)	C(-), A(V)	C(+)	C(-), A(+)	C(+), A(+)	C(-), A(+)	C(+)	C(-)	C(-)
Reduction of nitrates to nitrites	**L-tryptophane**	**D- glucose (Fermantation)**	**Urea**	**Esculine**	**4-nitrophenyl-b-D-galactopyra-noside**	**L-arabinose**	**Potassium gluconate**	**Capric acid**	**Adipic acid**	**Trisodium citrate**	**Phenyla-cetic acid**
C(+), A(-), G(-)	C(-), A(-), G(-)	C(-), A(+), G(+)	C(-), A(-), G(-)	C(+), A(+), G(+)	C(-), G(-)	C(-), A(-), G(-)	C(-), A(-), G(-)	C(-), G(-)	C(-), G(-)	C(-), G(-)	C(-), G(-)

C: *Chryseobacterium* sp. C-204; **A:** *C. aquaticum* KCTC 12483; **G:** *C. greenlandense* UMB34; **V:** Variable

Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility level was determined by minimum inhibitory concentration (MIC) testing against Oxytetracycline (OXY), Florfenicol, (FLO), Amoxicillin (AMO), Enrofloxacin (ENR), and Sulfamethoxazole-Trimethoprim (TRS) with 0.008 and 256 mg/L concentrations. *E. coli* ATCC 25922 was used as quality control (QC) strain (CLSI, 2014a, 2014b). After incubation at 22 °C for 48-72h, plates were measured at a wavelength of 595 nm in a microplate reader (Multiscan Go, Thermo), and MICs were defined as the lowest concentrations of antibiotic that inhibited growth.

Results

The C-204 isolate was isolated from the kidney in rainbow trout in the bacteriological sampling. The isolate was gram-negative, non-motile, oxidase, catalase, and flexirubin pigment positive. C-204 assimilated D-glucose, D-maltose, and D-mannose, but not the other substrates present on the API 20 NE strip, including L-arabinose, D-mannitol, N-acetyl glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid. The isolate hydrolyzed gelatin and esculin but did not hydrolyze 4-nitrophenyl-b-D-galactopyranoside (PNPG). It reduced nitrate to nitrite, but not produced indole, arginine dihydrolase. Furthermore, the production of acid from glucose was negative. All phenotypic characteristics of the closest two species and C-204 isolate are presented in Table 1. It was determined that the 16S rRNA sequence of C-204 isolate had similarities with the NCBI reference sequences in GenBank with *C. aquaticum* (99.65%) and *C. greenlandense* (98.95%).

MIC values of C-204 isolate were determined for FLO= 16 mg/L, OXY= 4mg/L, AMO= 16mg/L, TRS= 4/76mg/L and ENR= 0.064mg/L. The MIC values of the QC strain (*E. coli* ATCC 25922) were within the acceptable range recommended by the CLSI standards (CLSI, 2014b).

Table 2. Genomic characteristics of *Chryseobacterium* sp. C-204 isolate

Characteristics	**C-204**
GeneBank ID	JABSUA000000000
Genome Coverage	394.0X
Genome size (bp)	4012452
No. contigs	23
N50 contig size	362647
Largest contig size	954203
GC-content (%)	339
Total genes	3661
Protein-coding genes (CDS)	3590
tRNAs	65
Pseudogenes[a]	37

[a]The number of total Pseudogenes indicated includes genes with ambiguous residues, frameshifted gens, incomplete genes, genes with internal stopes, or other multiple problems.

Tablo 3. Putative AMR and virulence genes annotated in C-204 genome

Antimicrobial Resistance Gene		
Resistance Mechanism	**AMR Genes**	**Antimicrobial Class**
Antibiotic efflux	*tae*A, *oqx*B23, *oqx*B7, *tet*(60)	Pleuromutilin, Tetracycline, Glycylcycline, Diaminopyrimidine, Nitrofuran, Fluoroquinolone
Antibiotic inactivation	bla_{GOB-8}, bla_{CIA-1}	Penam, Cephalosporin, Carbapenem
Antibiotic target alteration	*mup*B	Mupirocin
Antibiotic target protection	*optr*A	Pleuromutilin, Tetracycline, Oxazolidinone, Lincosamide, Streptogramin, Macrolide, Fenikol
Virulence Genes		**Mechanism**
*eno, htp*B, *pgi, dna*K, *icl, KOX*_00005, *clp*C, *Car*B, *Zmp*1, *Ade*G, *Lig*A, *rpo*N, *clp*B		Adhesion, Invasion, Biofilm production, Proliferation in Macrophages, Escape from Phagocytosis, Inflammation activation and prevention of IL-1b production

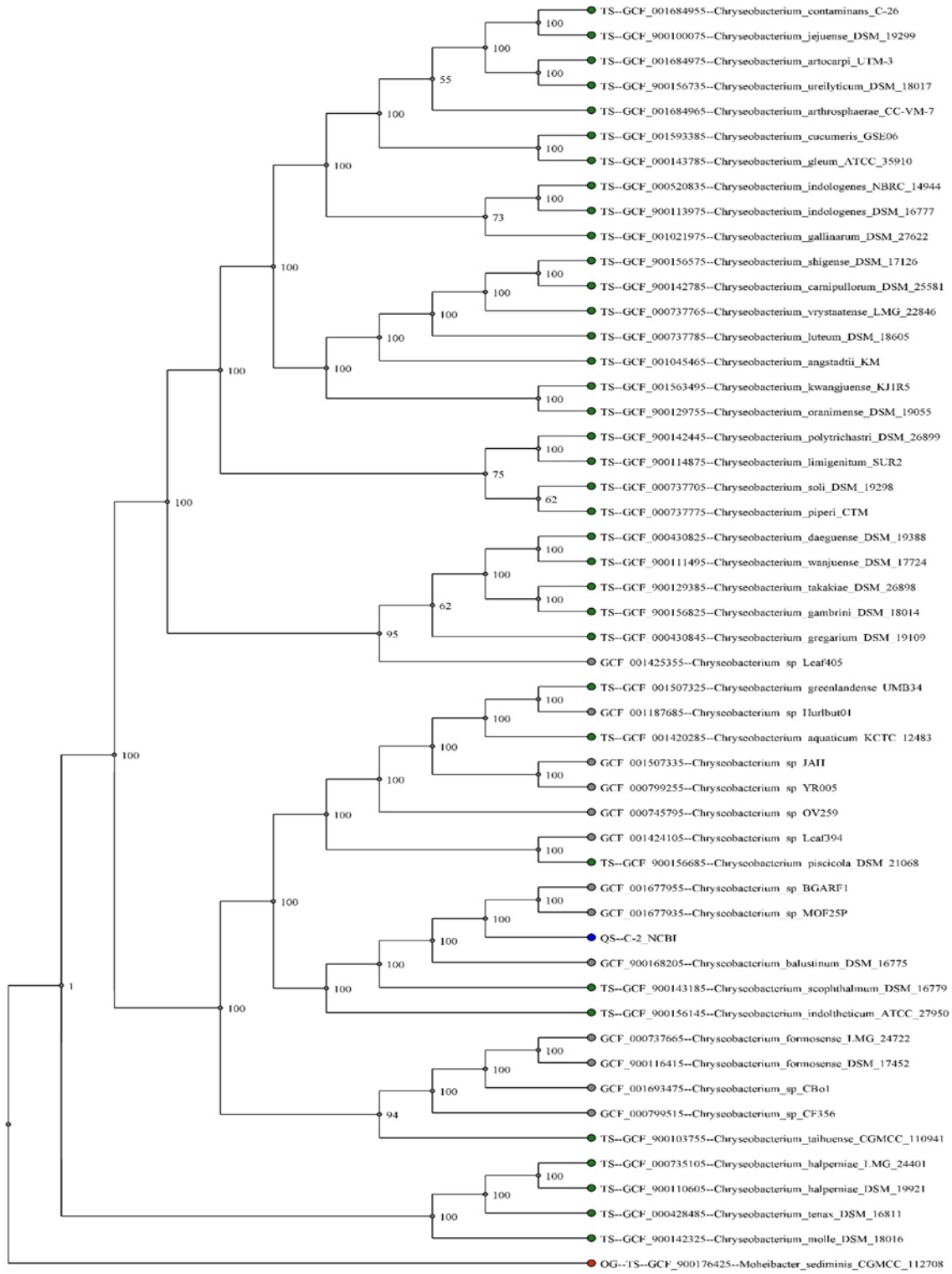

Figure 1. Phylogenetic tree of C-204 isolate (blue font) inferred from its draft genome using the software autoMLST. The phylogeny is rooted in *Moheibacter sedimnis CGMCC 112708* (red), strains depicted in green font represent *Chryseobacterium* type strains

204 genome after next-generation sequencing. The C -204 genome was assembled after filtering with BBDuk and using the SPAdes algorithm. After assembling, 23 contigs and 4.012.452 bp long genome were obtained. The GC ratio of the C-204 draft genome was found to be 33.9%. Genome characteristics of C-204 are presented in Table 2.

As a result of the autoMLST analysis performed with the closest 50 bacteria and 100 housekeeping gene regions, it was determined that the closest species to the C-204 isolate were *C. greenlandense* UMB34, *Chryseobacterium* sp. Hurlbut01 and *C. aquaticum* KCTC 12483 (Figure 1).

The draft genome of C-204 was registered with JABSUA000000000 accession number in GenBank. Eight antimicrobial resistance genes (AMR) and 13 virulence genes were detected in the genome of the C-204 isolate, and the related genes are presented in Table 3.

Discussion and Conclusion

While *Chryseobacterium* species do not cause any infection in domestic animals, it has been reported to cause infections in fish and frogs. The pathogenicity of *C. aahli, C. piscicola,* and *C. balustinum* in fish has been confirmed by experimental infection. Still, the pathogenicity of species such as *C. chaponense, C. piscum,* and *C. arothri*, which have been reported from systemic infections in fish in recent years, has not yet been confirmed. It has also been reported that some species within this genus, such as *C. indologenes*, are species with zoonotic potential that can cause diseases such as meningitis, urinary tract infections, and cystic fibrosis in humans (Hugo et al., 2019; Loch and Faisal, 2015a). At the same time, there is no study on the pathogenicity of *C. aquaticum* and *C. greenlandense* species in humans and fish.

In our study, detailed phenotypic characteristics of the C-204 isolate were determined by 106 different phenotypic tests (Biolog GEN III and API 20NE). It was seen that 19 of 66 tests were different from *C. aquaticum* type strain and 3 of 24 tests were different from *C. greenlandense* type strain. Also, 35 different tests that were not done before on these two types of strains were applied to the C-204 isolate for the first time. In shared biochemical tests (19 tests), C-204 isolate is differentiated from other type strains by nitrate reduction and production of acid from glucose. C-204 isolate, which was pre-identified as *Chryseobacterium* sp. by conventional tests, was found to be 99.65% similar to *C. aquaticum* and 98.95% *C. greenlandense* by 16S rRNA sequence analysis.

C. aquaticum was firstly isolated from healthy Siberian sturgeon juveniles in France, but the researchers reported the etiologic agent as *Chryseobacterium* sp. (Bernardet et al., 2005). It was later isolated from a water reservoir in Korea, and the species description was made (Kim et al., 2008). Also, this pathogen was reported from the rainbow trout in Iran with ulcerative erosion in the tail fin and peduncle area (Akhlaghi et al., 2012). Loch et al. (2013) isolated *C. aquaticum* from brown trout juveniles with endophthalmitis, discoloration, fin erosion, and kidney swelling. An experimental infection has not yet demonstrated the pathogenicity of this agent in studies.

C. greenlandense was isolated from ice obtained from 3.043 meters deep of the ice sheet in Greenland (Loveland-Curtze et al., 2010). In our study, according to the phylogenetic analysis performed with autoMLST, it was determined that the C-204 isolate was in the same genogroup as the *C. greenlandense* UMB34 and *C. aquaticum* KCTC 12483 strains. In Bergey's Manuel of Systematics of Archaea and Bacteri, it was suggested that *C. greenlandense* and *C. aquaticum* belong to the same species due to the high genome similarity and to be named *C. aquaticum*, which was first published (Hugo et al., 2019). Besides, García-López et al., (2019) reported that *C. greenlandense* is not a different species, it is a subspecies of *C. aquaticum*, and its naming should be made as "*Chryseobacterium aquaticum* subsp *greenlandense*" in the taxonomic classification created with the type strains in the Bacteroidetes phylum.

C-204 was isolated from the kidney of 1 gram rainbow trout with symptoms such as a dorsal lesion, loss of appetite, abnormal swimming, discoloration, and exophthalmos in a trout farm in the Aegean region. Since there is no scientific study on the pathogenesis of the determined *Chrysobacterium* species, the virulence genes in the genome of the C-204 isolatewere analyzed in detail. Thirteen different putative virulence genes encoding adhesion, invasion, biofilm production, macrophage reproduction, escape from phagocytosis, inflammation inactivation, and blocking of IL-1b production were found in the C-204 isolate genome (Liu et al., 2019). It is recommended to conduct experimental infection studies in rainbow trout to show whether the agent provides pathogenesis and Koch Postulate.

Antimicrobial drugs including florfenicol, oxytetracycline, amoxicillin, enrofloxacin and sulfadiazine/ trimethoprim have been licensed by the Ministry of Agriculture and Forestry to treat fish diseases in Turkey. The most commonly used antimicrobials in aquaculture are florfenicol, oxytetracycline, and sulfadiazine/trimethoprim. There are a lot of studies that have been reported on the development of antimicrobial resistance in bacteria isolated from aquaculture (Balta et al., 2010; Duman et al., 2017; Durmaz et al., 2012; Onuk et al., 2017; Saticioglu et al., 2019). There is no antimicrobial susceptibility determination and interpretation model for *Chryseobacterium* spe-

cies in EUCAST and CLSI standards. In this context, the antimicrobial susceptibility test protocol has been adapted according to previous studies on *Chryseobacterium* species. It has been reported that many agents in the *Flavobacteriaceae* family isolated from fish and clinical cases in humans can grow even at high concentrations of antimicrobials (Michel et al., 2005; Verner-Jeffreys et al., 2017). *C. indologenes* and *C. gleum* species isolated from clinical cases in humans have been naturally resistant to polymyxins, aminoglycosides (gentamicin, streptomycin, kanamycin), chloramphenicol, and most β-lactams (penicillins, cephalosporins, carbapenems) (Bellais et al., 2002; Lin et al., 2010). It has been reported that *C. scophthalmum* strains isolated in fish cases are resistant to tetracycline, aminoglycoside, lincomycin, oleandomycin, penicillin, and sulfadiazine in vitro, but are sensitive to chloramphenicol, sulfamethoxazole-trimethoprim, fusidic acid, and novobiocin (Mudarris and Austin, 1989). In our study, it was observed that C-204 isolate could grow even at high concentrations of Florfenicol, Oxytetracycline, Amoxicillin, and Sulphadiazine/Trimethoprim, which are licensed for fish. In the genome analysis, eight different antimicrobial resistance genes responsible for resistance against 15 different antibiotic classes were found. We concluded that the expression amount of the resistance genes detected in the genome of the C-204 isolate changes the phenotypic characteristic and makes the isolate resistant to antimicrobials.

This study is the first comprehensive study on *C. aquaticum* subsp *greenlandense* isolated from disease cases in rainbow trout. The phenotypic characteristics of the agent were determined for the first time using 106 different tests.

It has been determined that the C-204 isolate can grow even at high concentrations of antimicrobials commonly used in aquaculture and carry resistance genes against these antimicrobials in the genome.

It has been determined that there are 13 different virulence genes in the genome of the C-204 isolate, and these virulence genes may be responsible for the development of disease in rainbow trout.

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