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Draft genomes of *Nautella italica* strains CECT 7645^T and CECT 7321: Two roseobacters with potential pathogenic and biotechnological traits

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ABSTRACT

Nautella italica is a member of the family *Rhodobacteraceae* described in 2009. Strain LMG 24365^T (= CECT 7645^T, = DSM 26436^T, = CCUG 55857^T) was isolated from a marine electroactive biofilm growing in a stainless steel cathode exposed to natural water in Genoa, Italy. Strain AD 41 (= CECT 7321) was isolated from water surrounding cultivated gilthead seabream larvae in Cádiz, Spain. The genomes of strains CECT 7645^T and CECT 7321 were sequenced, assembled, annotated and compared. Here we describe the most relevant findings: biofilm formation, quorum sensing, resistance to multiple drugs, heavy metals and oxidative stress, cytotoxins, and poly-β-hydroxybutyrate (PHB) production genes. These genomes were also compared to current available genomes in NCBI Genome Database from members of the genus *Nautella*, *Nautella* sp. R11 and *Nautella* sp. ECSMB14104. The comparison showed a higher similarity between strains CECT 7645^T and R11 compared to strain CECT 7321 and strain ECSMB14104. The genome similarity indexes allowed confirming and assigning strains CECT 7321, R11 and ECSMB14104 to the species *N. italica*.

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1. Introduction

Nautella is a genus of the family *Rhodobacteraceae*, order *Rhodobacterales* within the class *Alphaproteobacteria.* It also belongs to the so-called *Roseobacter* group, which is one of the most ubiquitous and predominant components of marine bacteria and plays an important role in biochemical global processes (Pujalte et al., 2014). *Nautella italica* was described by Vandecandelaere et al. (2009), and up to now it remains the only species described in this genus.

N. italica LMG 23465^T was isolated, together with other 4 isolates belonging to the same species, from a marine electroactive biofilm grown on a stainless steel cathode exposed to natural seawater at the ISMAR-CNR Marine Station located in the port of Genoa, Italy (Vandecandelaere et al., 2009). It is gram-negative, moderately halophilic, strictly aerobic, catalase and oxidase-positive with a growth temperature range from 4 to 45 °C (Table 1). Cells are motile by a polar flagellum, rod-shaped (0.7–0.77 × 1.5–2.1 μm), and contain poly-ß-hydroxybutyrate (PHB) inclusion bodies. Colonies on Marine Agar are beige, round and 1–2 mm diameter after 3 days of incubation, with smooth surface and convex with entire margins. They cannot reduce nitrate to nitrite and do not ferment glucose or produce indole.

N. italica CECT 7321 was isolated from sea water surrounding gilthead seabream larvae (*Sparus aurata*) in Cádiz, Spain in 1998 (Pujalte et al., 2003). Information about this strain is also contained in Table 1.

Prior to this study the only genome and assembly available at the NCBI Genome Database within the genus *Nautella* was that of strain ECSMB14104, isolated from a glass biofilm in East China Sea. Another isolate, strain R11, causing bleaching disease in marine alga *Delisea pulchra* (isolated from southern Australia) was first assigned to the genus *Ruegeria* (Case et al., 2011), and then to *Nautella* as it has 100% 16S rRNA gene similarity with *N. italica* LMG 24365^T (Fernandes et al., 2011). Its genome was described in the later study and showed several characteristics related to pathogenic abilities.

Marine bacteria are a well-known genetic resource for bioremediation, biotechnology and biomedicine applications (Yakimov et al., 2007; Eom et al., 2013; Kiran et al., 2014). Thus, *de novo* genome sequencing, like the present study, or metagenomic bioprospecting studies of marine bacteria (Kodzius and Gojobori, 2015) provide relevant information about novel genes, pathways and genomes with important applications.

We present here the draft genomes of two strains of *N. italica*, CECT 7645^T and CECT 7321 (Table 1), together with its annotation and comparative study. Both genomes reveal potential pathogenic factors as previously described for *Nautella* sp. R11 (Fernandes et al., 2011; Case et al., 2011).

Abbreviations: PHB, poly-β-hydroxybutyrate.

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Table 1
Classification and general features of *N. italica* strains CECT 7645^T and CECT 7321.

Property	*N. italica* CECT 7645^T	*N. italica* CECT 7321
Classification	Domain *Bacteria* Phylum *Proteobacteria* Class *Alphaproteobacteria* Order *Rhodobacterales* Family *Rhodobacteraceae* Genus *Nautella* Species *Nautella italica* (Type) strain: CECT 7645^T = CCUG 55847^T = DSM 26436^T = LMG 24365^T = strain 11^T = Vandecandelaere R-26144^T	Domain *Bacteria* Phylum *Proteobacteria* Class *Alphaproteobacteria* Order *Rhodobacterales* Family *Rhodobacteraceae* Genus *Nautella* Species *Nautella italica* Strain: CECT 7321 = AD 41
Gram stain	Negative	Negative
Cell shape	Rods	Rods
Motility	Motile	Motile
Sporulation	Not reported	Not reported
Temperature range	4–45 °C	15–45 °C
Optimum temperature	20–28 °C	26 °C
pH range; optimum	5.5–9.0; 6.5–8.0	Not determined
Carbon source	Unknown	D-glucose, D-fructose, D-galactose, glycerol, D-mannitol, *m*-inositol, pyruvate, acetate, propionate, citrate, 2-oxoglutarate, succinate, fumarate, lactate, 3-hydroxy-butyrate, L-glycine, L-threonine, L-glutamate, L-alanine, L-tyrosine, L-sarcosine
Habitat	Marine	Marine
Salinity	36‰ NaCl (w/v)	33‰ NaCl (w/v)
Oxygen requirement	Strictly aerobic	Strictly aerobic
Biotic relationship	Free-living	Free-living
Pathogenicity	Not reported	Not reported
Geographic location	Italy	Spain
Sample collection	2005	1998
Latitude	44° 24′36″N	36° 28′33.6″N
Longitude	8° 54′00″E	6° 11′2.4″W
Altitude	0 m	0 m

2. Materials and methods

2.1. Genomic DNA extraction and sequencing

N. italica CECT 7645^T and CECT 7321 were cultured in marine agar (MA; Difco) at 26 °C under aerobic conditions during 3 days. Genomic DNA was isolated using Real Pure Spin kit (Durviz) following the standard protocol recommended by the manufacturer. The integrity of the extracted DNA was checked by visualization in a 2.0% agarose gel electrophoresis. Its purity and quantity was checked by measuring the absorbance at 260 and 280 nm with a spectrophotometer Nanodrop 2000c (Thermo Scientific) and calculating the ratio A260/A280.

The genomes of *N. italica* CECT 7645^T and CECT 7321 were sequenced at Central Service of Support to Experimental Research (SCSIE) of the University of Valencia (Valencia, Spain) using an Illumina Miseq technology with 2 × 250 paired-end reads.

2.2. Genome assembly and annotation

The Illumina reads were analyzed for quality control using FASTQC, a common quality control tool developed by Babraham Bioinformatics to check raw sequencing data. After filtering using different NGS manipulation tools – based on overall quality, positional quality and length of the reads – the remaining reads were assembled using SPADES 3.0.0 de novo assembler (Bankevich et al., 2012). All these tools are wrapped in Galaxy Orione Server (Cuccuru et al., 2014).

The draft genomes were annotated using Prokka (Seemann, 2014) an open source software tool, within Galaxy Orione Server, and using RAST v2.0 (Rapid Annotation using Subsystem Technology) (Aziz et al., 2008). Signal peptides were searched using SignalP 4.1 Server (Petersen et al., 2011). CRISPR repeats were examined by CRISPRFinder (Grissa et al., 2007). Transmembrane helix domains were predicted through TMHMM server v.2.0 (Krogh et al., 2001). Protein coding genes were analyzed for COG functional annotation using WebMGA server (Wu et al., 2011). Secondary metabolites were predicted using antiSMASH 2.0 (Medema et al., 2011). Pfam domains were predicted using NCBI Batch CD-Search Tool (Marchler-Bauer et al., 2015) using default parameters.

2.3. Phylogeny study

The 16S rRNA gene sequence similarity was studied with EzTaxon (Kim et al., 2012) using SSU rRNA sequences. A phylogenetic tree was obtained through alignment of SSU rRNA gene sequences with version 123 of the 'All-Species Living Tree' project SSU rRNA gene database (Yarza et al., 2008) using the ARB software package (Ludwig et al., 2004). The phylogeny was constructed from nearly full-length gene sequences using the neighbor-joining method (Saitou and Nei, 1987) within ARB, filtered to exclude alignment positions that contained gaps or ambiguous nucleotides in any of the sequences included in the tree.

2.4. Genome deposit in genomic database

The draft genome sequencing projects of *N. italica* CECT 7645^T and *N. italica* CECT 7321 were registered in the European Nucleotide Archive database under accession numbers ERP010491 and ERP010490 respectively, together with the sequence read archives ERR894644 and ERR894505, and annotated contigs CVRM01000001-CVRM01000037 and CVRL01000001-CVRL01000046.

2.5. Genomic similarity calculation

Genomic similarity indexes were calculated using genome assembly contig fasta files of *N. italica* CECT 7645^T, *N. italica* CECT 7321 and *Nautella* sp. ECSMB14104 and fasta files of *Nautella* sp. R11 chromosome

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