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Draft genome sequence of *Psychrobacter* sp. ENNN9_III, a strain isolated from water in a polluted temperate estuarine system (*Ria de Aveiro*, Portugal)

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ABSTRACT

The genus *Psychrobacter* includes Gram-negative coccobacilli that are non-pigmented, oxidase-positive, non-motile, psychrophilic or psychrotolerant, and halotolerant. *Psychrobacter* strain ENNN9_III was isolated from water in a polluted temperate estuarine system, contaminated with hydrocarbons and heavy metals. The genome has a G + C content of 42.7%, 2618 open reading frames (ORFs), three copies of the rRNAs operon, and 29 tRNA genes.

Twenty-five sequences related to the degradation of aromatic compounds were predicted, as well as numerous genes related to resistance to metals or metal(loid)s. The genome sequence of *Psychrobacter* strain ENNN9_III provides the groundwork for further elucidation of the mechanisms of metal resistance and aromatic compounds degradation. Future studies are needed to confirm the usefulness of this strain for bioremediation proposes.

1. Introduction

Genus *Psychrobacter* belongs to the family *Moraxellaceae* [1], and includes Gram-negative, non-pigmented, oxidase-positive, cold-adapted, halotolerant, strictly aerobic organisms. Members of this genus are particularly successful in cold environments and have been isolated from diverse sources including permafrost soil, sea ice, seawater, fish and processed food [2,3]. *Psychrobacter* strains are capable of growth at temperatures between −10 °C and 42 °C [3,4]. The genus currently comprises 35 validly described species (http://www.bacterio.net/). The complete genome sequences of two strains of *Psychrobacter arcticus* [5] and *Psychrobacter cryohalolentis* [6] were reported. Both strains were isolated from permafrost soil in Siberia and are capable of growth at −10 °C.

Strain *Psychrobacter* ENNN9_III was isolated from water in *Ria de Aveiro*, Portugal [7,8]. This polluted temperate estuarine system receives industrial and urban effluents, and is also subjected to runoffs from agricultural fields and aquacultures [8]. Some areas of the estuary are considerably contaminated with heavy metals, particularly mercury and arsenic, due to the discharge of metal-containing industrial effluents for several decades [9]. A relevant contamination with hydrocarbons has also been detected in *Ria de Aveiro* [10], probably related to harbor facilities settled in the estuary.

2. Results and discussion

Genome sequence of *Psychrobacter* sp. ENNN9_III provided 147,696,952 and 1,957,796 reads SOLiD 5500xl and the Ion Torrent PGM respectively, and as the resulted in a final assembly of 2,988,999 bp in 210 contigs (with N50 of 19,123 bases) (Table 1), suggesting a genome size similar to the ones reported for the *Psychrobacter* strains previously sequenced [5,6]. The genome has an average G + C content of 42.7% and contains approximate 2618 open reading frames (ORFs), three copies of the 16S, 23S, and 5S rRNA genes and 29 tRNA genes (Table 1). In terms of gene

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Table 1
General features of *Psychrobacter* sp. ENNN9_III draft genome.

Attributes	Values
Genome size (bp)	2,988,999
Total numbers of contigs	210
Contigs N50 (bp)	19,123
ORFs	2618
G + C content %	42.7%
tRNA	29
rRNA	9
Data accessibility	The draft genome sequence has been deposited in Genbank, under the accession number LNUO00000000.

synteny, *Psychrobacter* sp. ENNN9_III was more closely related to *Psychrobacter cryohalolentis* (GCA_000013905).

The complete sequence of the 16S rRNA gene of strain ENNN9_III included 1527 nt. This sequence shared 98% to 100% similarity with type strains of the genus *Psychrobacter*. In a phylogenetic tree including *Psychrobacter* species type strains, strain ENNN9_III clustered together with *Psychrobacter aquimaris*, *Psychrobacter muriicola and Psychrobacter nivimaris* (Fig. 1).

Twenty-five sequences related to the degradation of aromatic compounds were predicted, including genes for enzymes like catechol dioxygenase, benzoate and protocatechuate. Aromatic compounds can be aerobically degraded by two different pathways, originating catechol and protocatechuate [11]. According to Blast2GO annotation, the benzoate degradation via hydroxylation pathway is present in the genome of *Psychrobacter* sp. ENNN9_III (Fig. 2) and *Psychrobacter cryohalolentis*. Catechol is a common intermediate in this pathway and this compound may be oxidized by the ortho or meta cleavage pathways. Annotation of the *Psychrobacter* sp. ENNN9_III genome indicates the presence of the ortho cleavage pathway originating Acetyl-CoA and Succinyl-CoA, which can be subsequently used in Krebs cycle [12].

The protocatechuate degradation pathway, although not present in the two *Psychrobacter* genomes currently available (*Psychrobacter cryohalolentis* and *Psychrobacter arcticus*), was found in the *Psychrobacter* sp. ENNN9_III genome, suggesting a good adaptation of this strain to hydrocarbons-contaminated environments.

In previous studies, the ability of *Psychrobacter* strains to degrade hydrocarbons has been suggested [13,14]. The use of hydrocarbons as the sole carbon source was assessed for *Psychrobacter* sp. ENNN9_III in mineral medium supplemented with diesel oil [8]. However the strain was not able to grow in these conditions. We can speculate that the complex hydrocarbon mixture used in these experiments may contain substances able to inhibit the growth of this strain. The lack of expression of some of the degrading genes may also explain this result.

The analysis of the genome also allowed to identify numerous genes related to resistance to metals or metal(loid)s. For example, the operon *ars*, which may confer resistance to arsenic, was identified and included the genes *arsA* (encoding a pump-driving ATPase), *arsB* (encoding an arsenite efflux pump), *arsC* (a detoxifying arsenate reductase gene), *arsH* (encoding a protein with unknown function that contributes to arsenic resistance) and *arsR* (encoding a regulatory protein). These genes were identified in scaffolds 52, 91, 92 and 130 and the corresponding deduced aminoacid sequences shared 81 to 98% similarity with proteins previously identified in *Psychrobacter* genomes.

Arsenic contamination in coastal areas, including *Ria de Aveiro*, has been reported mainly in the forms of arsenite or arsenate, both forms being toxic to living organisms [9,15]. Liao and co-workers [16] confirmed for the first time the ability of *Psychrobacter* strains to oxidize arsenite or reduce arsenate, and thus survive in arsenic-contaminated water. The repertoire of putative arsenic resistance genes detected in *Psychrobacter* sp. ENNN9_III suggests that this strain uses a mechanism

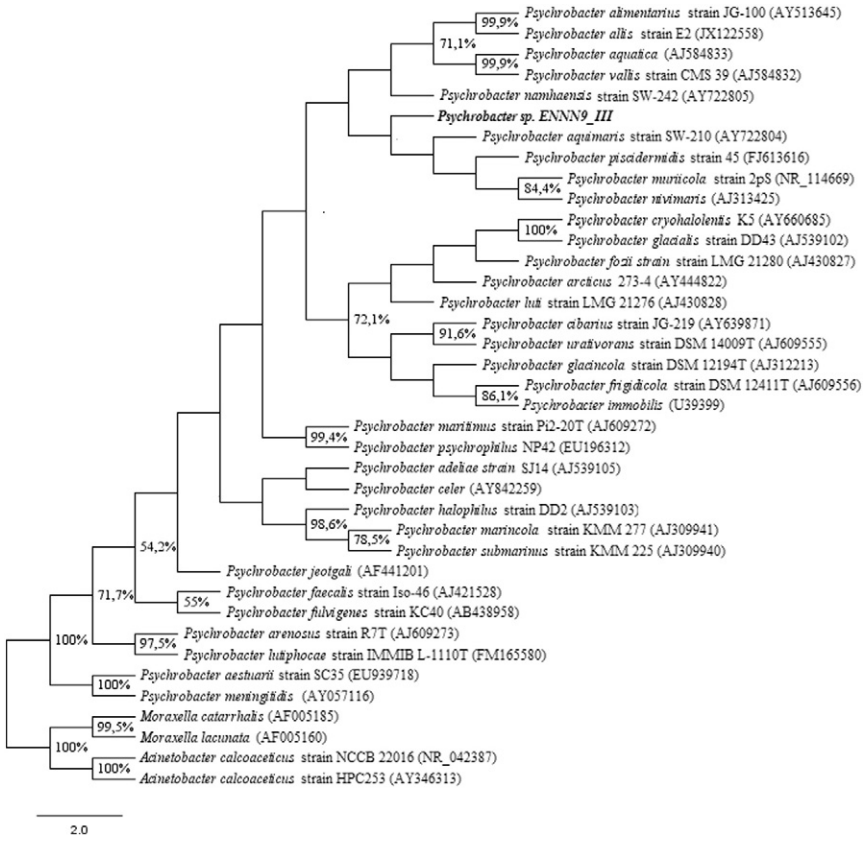
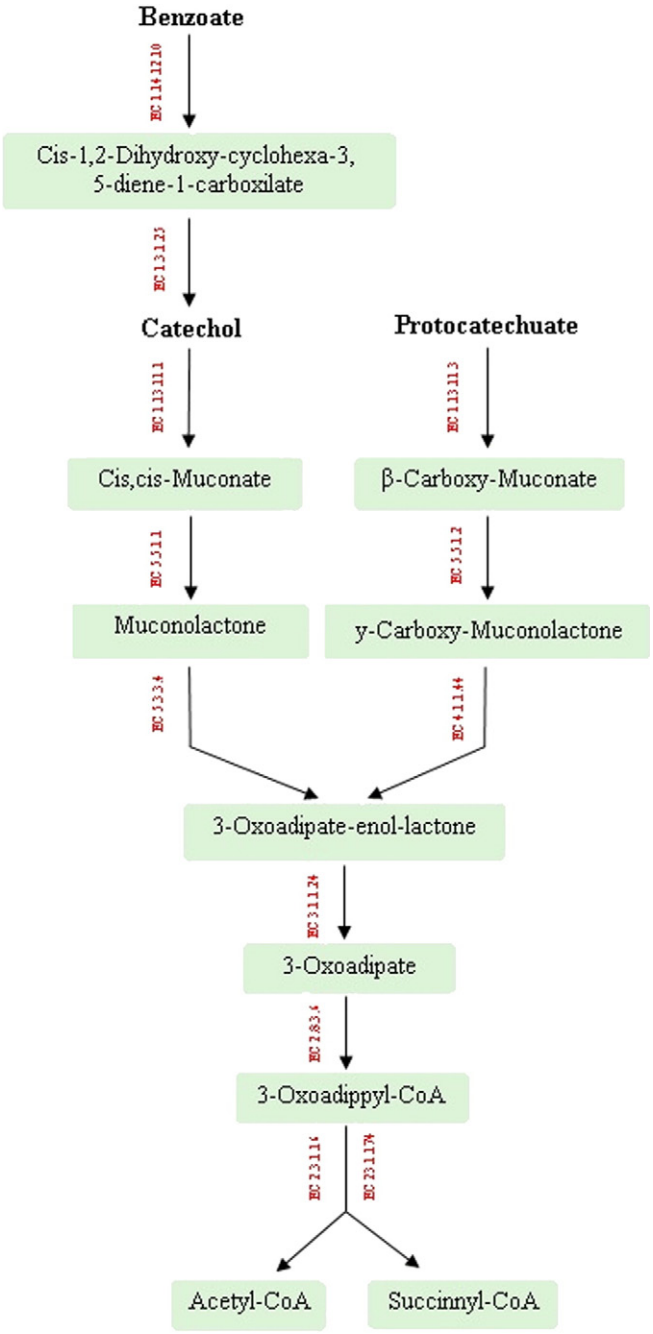


Fig. 1. Phylogenetic tree of gene 16S rRNA of the genus *Psychrobacter*. 16S rRNA gene-based phylogenetic tree highlighting the position of *Psychrobacter* sp. ENNN9_III (in bold) relative to the type strains of the other species within the genus *Psychrobacter*.



EC numbers	Enzyme	Contig
EC 1.13.11.1	1,2-dioxygenase	71
EC 1.13.11.3	3,4-dioxygenase	100
EC 5.5.1.1	cycloisomerase	71
EC 5.5.1.2	cycloisomerase	172
EC 5.3.3.4	Delta-isomerase	71
EC 4.1.1.44	decarboxylase	160
EC 3.1.1.24	Enol-lactonase	39
EC 2.8.3.6	CoA-transferase	53
EC 2.3.1.16	C-acyltransferase	117
EC 23.1.174	CoA thiolase	48/53

of arsenite extrusion for cytoplasm defense to survive in contaminated environments.

3. Conclusions

The analysis of the genome sequence of *Psychrobacter* sp. ENNN9_III adds further insights into the mechanisms that strains of this genus may use to cope with pollutants and thus proliferate in contaminated environments. Although genes conferring metal resistance or related to hydrocarbon degradation were identified in previously reported *Psychrobacter* genome sequences, the genome here analyzed is the one that comprises a more diverse repertoire of these genetic determinants. More studies are needed to confirm a future usefulness of this strain for bioremediation purposes.

4. Material and methods

4.1. SOLiD and ion torrent PGM sequencing

Genome sequence of *Psychrobacter* sp. ENNN9_III was obtained by using a combined approach with the SOLiD 5500xl (Life Technologies) and the Ion Torrent PGM (Life Technologies) platforms. Both sequencing approaches were based on paired-ended libraries. Quality of raw reads was assessed with the FastQC software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and the bases with quality value bellow Phred 20 were removed.

4.2. Genome assembly and annotation

Draft genome was assembled using the packages Velvet [17], MIRA [18] and SPAdes [19]. CISA [20] and Lasergene v11 Suite (DNAStar) software were used to reduce the number of contigs. Subsequently, the Mauve software [21] was applied to order the draft genome by using the genomes of *Psychrobacter cryohalolentis* [6] and *Psychrobacter arcticus* 273-4 [5] as references.

The Gepard software was used for synteny analysis [22]. The automatic annotation was used by RAST — Rapid Annotations using Subsystems Technology [23]. The rRNA and tRNA were predicted by RNAmmer [24] and tRNAscan-Se [25], respectively. Predicted coding sequences (CDSs) were annotated using Blast2GO [26]. Functional classification of CDSs was performed based on Gene Ontology databases.

4.3. Phylogenetic analysis

The tree was generated based on the 16S rRNA gene sequences using the Maximum Likelihood method based on the General Time Reversible model in MEGA v6 software [27]. Bootstrap values greater than 50% are shown above the node of each main group. Sequences of the strains *Moraxella catarrhalis* (AF005185), *Moraxella lacunata* (AF005160), *Acinetobacter calcoaceticus* strain NCCB 22016 (NR_042387) and *Acinetobacter calcoaceticus* strain HPC253 (AY346313) were used as outgroup. The scale bar corresponds to the nucleotide substitution rate.

Conflict of interest

The authors have declared that no competing interests exist.

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Fig. 2. Schematic representation of benzoate degradation pathway. Catechol and protocatechuateare converted in Krebs cycle intermediates, via ortho cleavage pathway; enzymes are represented by identification codes (EC numbers).

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