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Short Communication

Genome of the epiphytic bacterium *Achromobacter denitrificans* strain EPI24, isolated from a macroalga located in the Colombian Caribbean

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ABSTRACT

Marine macroalgae are being recognized as reservoirs of biologically active compounds, as their surfaces are susceptible to the colonization of microorganisms which can produce enzymes with a wide range of molecular architectures. Among these bacteria, *Achromobacter* is responsible for the biosynthesis of laccases. In this research, we performed a bioinformatic pipeline to annotate the sequenced complete genome of the epiphytic bacterium *Achromobacter denitrificans* strain EPI24, from the macroalgal surface of the *Ulva lactuca* species; this strain showed laccase activity which has been previously assessed on plate assays. The genome of *A. denitrificans* strain EPI24 has a size of ~6.95 Mb, a GC content of 67.33%, and 6,603 protein-coding genes. The functional annotation of the *A. denitrificans* strain EPI24 genome confirmed the presence of genes encoding for laccases, which could have functional properties of interest in processes such as the biodegradation of phenolic compounds under versatile and efficient conditions.

1. Introduction

Currently, phenolic dyes are an environmental problem owing to recalcitrance. These important and diverse xenobiotic compounds have been used in the textile industry because of efficiency and low cost. However, the inappropriate disposal of wastewater is a big challenge that disturbs all aquatic environments in the whole world. Wastewater containing phenolic compounds affects humans and other organisms for their high toxicity [1,2].

Achromobacter has been described as a potential bioremediation agent of industrial wastewater and soils containing minerals, salts, polycyclic aromatic, and phenolic compounds [3]. The degradation of phenolic dyes implies the breakdown of the aromatic ring by oxidases such as laccases along with other enzymes. Some lacasse type enzymes such as polyphenol oxidase (PPO) and Multicopper oxidase (MCO) have been associated with physiological response to toxic levels of heavy metals and tolerance to oxidative stress [4]. Although fungal and some bacterial laccase types have been investigated, those of marine origin could be more interesting in different biotechnological applications due to their wide versatility and physicochemical and kinetic properties such as salt tolerance and thermostability, etc.; inherent advantages of the changing conditions in the marine ecosystems [5].

Achromobacter belongs to the *Alcaligenaceae* family, order Burkholderiales of the class β-Proteobacteria [6]. This genus is obligately aerobic, asporogenous, chemo-organotrophic and comprises 19 officially recognized species [7]. *Achromobacter denitrificans* is a gram-negative bacterium of 0.8–1.2 × 2.5–3.0 μm in size, motile by peritrichous flagella. A non-fermenting, oxidase- and catalase-positive bacterium. *A. denitrificans* grows at 30 °C, in LB medium and marine agar. This bacterium has been found in a wide variety of environments ranging from soil to water and may be part of the microbiota of the ear and the gastrointestinal and respiratory tracts in some people [8].

Studies of epiphytic bacteria from marine macroalgae of the *Ulva* genus identified *Vibrio, Pseudomonas* and *Achromobacter* genera as representatives of the culturable bacterial community from this macroalga [9]. Regarding, recent studies have described Gram negative bacteria of the genera *Pseudoalteromonas, Shewanella, Halomonas, Roseobacter, Marinomonas,* and *Vibrio* and some Gram positive bacteria such as *Bacillus* genus, as part of the culturable epiphytic component from *Ulva* [10–14]. In this study, we obtained a strain belonging to the *Achromobacter* sp., being the first record of this genus within the bacterial epiphytes found in *U. lactuca* sampled in the Colombian Caribbean Sea. This

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work aims to present the draft genome of the *U. lactuca* epiphyte *A. denitrificans* strain EPI24, focusing on genes involved in the laccase activity to highlight on its potential application in the biodegradation process of phenolic compounds as dyes.

2. Data description

The *A. denitrificans* strain EPI24 was isolated from the *U. lactuca* surface. The raw algal material was searched in an area of rockyshore "La Punta de la Loma", Santa Marta – Colombia, in the intertidal zone, 10 km south of the river Gaira. EPI24 was inoculated according to the methodology proposed in previous research [11] (Table 1). The bacterium was cultivated in LB agar and incubated at 30 °C for 48 h. The agar plate screening for positive laccase production was performed with three assays with different phenolic substrates (guaiacol, 2,6-Dimethoxyphenol and tannic acid) as described by Dhiman [15].

Previous studies have shown some strains of the *Achromobacter* sp. as lacasses producers, these strains were isolated from contaminated sediments or agro-industrial residues [16,17]. *A. denitrificans* strain EPI24 was chosen for genome sequencing to supply more information on laccases-producing marine epiphytic bacteria. The genomic DNA of *A. denitrificans* EPI24 was isolated with the gBac Mini DNA Bacteria Kit (IBI SCIENTIFIC®). The resulting DNA was sequenced making use of the Illumina paired-end MiSeq platform [18]. A total of 1444,648 paired-end reads were obtained and filtered with the Trimmomatic v0.38 tool [19]. Once the low-quality reads were removed, the reads with the required quality were assembled with SPAdes v3.13.1 [20], using the strain *A. denitrificans* NCTC8582 (ID GCA_013343095.1_ASM1334309v1) as reference genome.

The *A. denitrificans* EPI24 complete genome includes one circular chromosome of 6943,456 bp (6.94 Mb), GC content equivalent to 67.33% and 6603 CDSs (Fig. 1.). The assignment of this strain to a species of genus *Achromobacter* was evaluated with two genome-to-genome approaches for the sequence comparison: The first was the average nucleotide identity (ANI) analysis with the EzGenome ANI web resource (http://www.ezbiocloud.net/ezgenome/ani) [21] and the second the TCS parameter (Tetra correlation score) evaluated with the JSpeciesWS server [22]. Our results are consistent with the size and GC content submitted in the genomes of *Achromobacter* sp. (genome size of 6.24 Mb, GC content of 64.8% and 5578 CDSs for *Achromobacter* sp. B7 and genome size of 6.91 Mb, GC content of 67.0% and 6390 CDSs for *A. xylosoxidans* NH44784–1996) [23,24]. Differences in the number of predicted CDSs may be due to the sequencing method, number, quality of the reads and the bioinformatic pipeline used.

Table 1
General features and genome sequencing project information of *Achromobacter denitrificans* strain EPI24 according to the MIGS recommendations.

Item	Description
General feature	
Classification	Domain Bacteria Phylum Proteobacteria Class Betaproteobacteria Order Burkholderiales Family Alcaligenaceae Genus *Achromobacter*
Gram stain	Negative
Cell shape	Short rod
Investigation	
Investigation type	Bacteria
Project name	Genome sequencing of *Achromobacter denitrificans* EPI24
Collection date	23/11/2019
Geographic location (Latitude and location)	11°07′00′′N 74°14′01′′W
Geographic location (Country and/or sea, region)	Colombia: Santa Marta
Environment (biome)	Caribbean sea
Environment (material)	*Ulva lactuca*
Biotic relationship	Epiphytic bacterium
Trophic level	Chemoorganotroph
Relationship to oxygen	Aerobe
Sequencing	
Sequencing method	Illumina MiSeq
Assembly	Spades
Finishing strategy	Complete; 56,32 X coverage

A number of 6603 protein-coding genes were predicted with the functional annotation performed by the PATRIC/RAST platforms [25]. Of them, 1387 (21%) were classified as hypothetical proteins, 57 tRNA and 11 rRNA. The Gene functional categories were identified based on the cluster of orthologous groups (COG) by the Eggnog Mapper Platform [26]. The most important categories corresponded to energy production and conversion (C), amino acid transport and metabolism (E), transcription (K), metabolism and transport of inorganic ions (P) and metabolism and transport of lipids (I) (Fig. 1.). Genes assigned to the inorganic ion transport and metabolism category (6.4%) could be involved in the homeostasis of essential ions that serve as prosthetic groups of different enzymes like laccases and cytochromes. In addition, there is evidence for biphenyl catabolism, arsenite oxidation, haloaromatic acid degradation, detoxification of chromium, and hydrocarbon degradation by members of the *Achomobacter* genus [27].

The functional annotation predicted two gene sequences that could be involved in the laccase activity previously evidenced in plate assays [15]. The expression of laccase-type enzymes can be explained by the presence of natural phenolic compounds as lignin and antioxidants on the macroalgal surface [28]. Previous studies have shown that the macroalgae cell wall contain lignin in a low proportion (5,5 – 6,7%) [29–31]. In addition, these compounds can also be present in the marine environment as pollutants because of anthropic activities [32]. In the Santa Marta region studies reported the presence of dissolved hydrocarbons and hydrocarbon polycyclic aromatics [33]. In addition, *Ulva* has been used as a low-cost absorbent for removing phenolic compounds in polluted environments [34,35].

The *in-silico* analysis of gene sequences identified a product of 587 amino acids predicted as multicopper oxidase (MCO) (100% of identity) and a sequence of 242 amino acids corresponding to a multicopper polyphenol oxidase (PPO) (99.21% of identity). All bacteria present a diversity of strategies to sense and respond to environmental stressor agents. The identification of these enzymes can be explained by inherent mechanisms to overcome the presence of heavy metals, toxic compounds and maintain copper homeostasis [36,37]. Besides, these enzymes may provide protection from the oxidizing stress caused by the generation of reactive oxygen species (ROSs) by *Ulva* to prevent the colonization of pathogen microorganisms [38,12].

According to the CDD database, the multicopper oxidase belongs to the copA family [39]. This family group laccases and L-ascorbate oxidases, which showed a typical signal sequence known as TAT (twin arginine translocation signal). copA family enzymes cross the inner membrane through cofactors that bind metals, as well as copper-binding motifs. In functional terms, these enzymes play important roles in detoxification, transport and binding of proteins, cations and iron-carrying compounds [40].

The results of the multiple alignment performed with the amino acid sequences of multicopper oxidases showed the presence of aminoacidic motifs for the binding of tri nuclear copper, which start in a histidine residue (H101) and end in another histidine residue (H145). In this region, the interaction between the ligand and the copper takes place. The following two conserved regions correspond to interface domains [41], where internal interactions occur (Fig. 2.).

The putative domain interface 3 starts in a histidine residue (H101) and ends in a glutamine residue (Q151), while the putative interface domain 2 starts in a phenylalanine residue (F48) and ends in a valine residue (V159). The multiple alignment also showed the twin arginine translocation domain (RR), which is characteristic in periplasmic proteins that cross the inner membrane. This domain could have metal-containing cofactors or copper-binding sites [39].

The genomic analysis of the epiphytic bacterium *A. denitrificans*

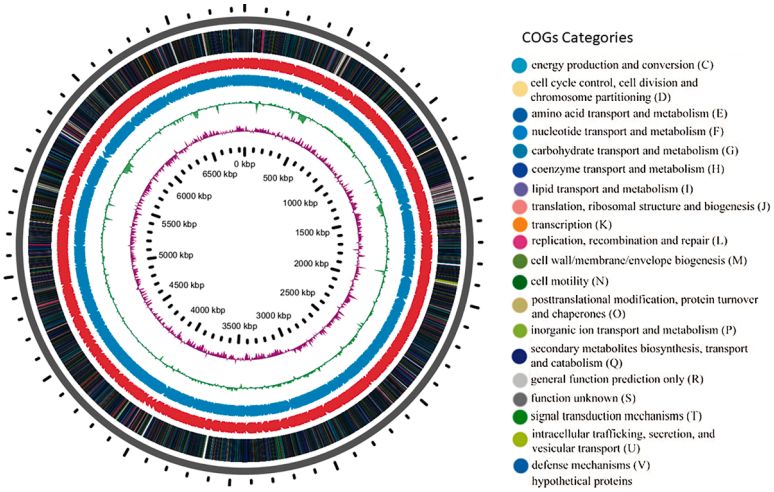

Fig. 1. Circular representation of the *Achromobacter denitrificans* EPI24 genome. From the outside to the center: The scale, chromosome (gray), COG categories (different colors described in the right chart), CDSs on forward strand (red), CDSs on reverse strand (yellow), GC content (black) and GC skew (purple).

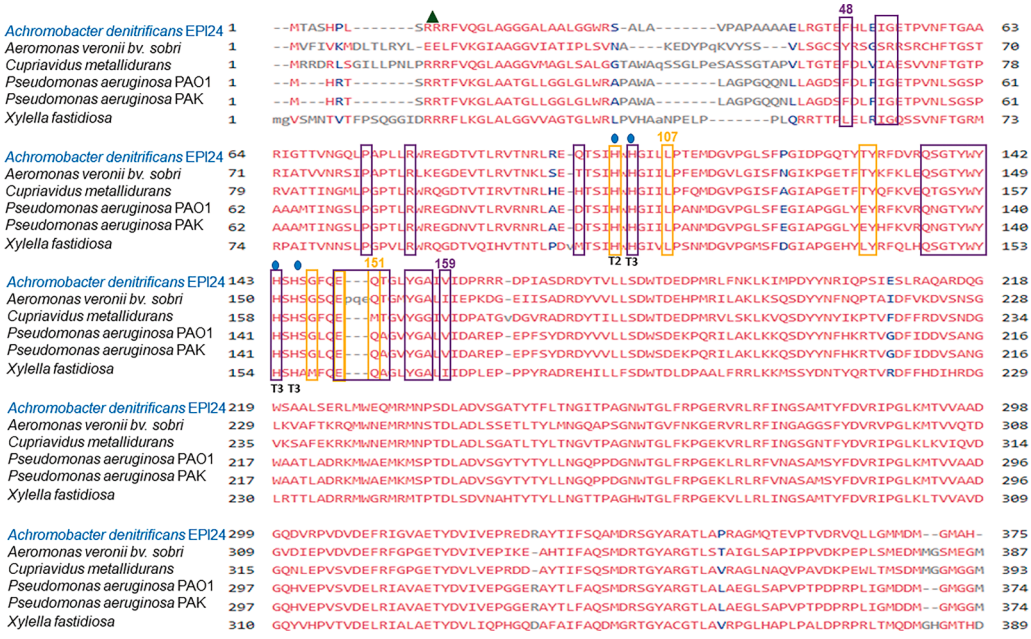

Fig. 2. Multiple amino acid sequence alignment of multicopper oxidases from *Achromobacter denitrificans* strain EPI24 and other species. The amino acid sequences used were: predicted multicopper oxidase from *A. denitrificans* EPI24, AAF32269.1 from *Aeromonas veronii bv. Sobri*, CAC07979.1 from *Cupriavidus metallidurans* CH34, NP_250,755.1 from *Pseudomonas aeruginosa* PAO, AAN52530.1 from *Pseudomonas aeruginosa* PAK and AAO27999.1 from *Xylella fastidiosa*. Amino acids highlighted in red are conserved, amino acids in blue are modifications and blue circles represent copper binding sites. Frames correspond to: a putative interface domain 2 (polypeptide binding site) colored violet, interface domain 3 colored yellow. The black triangle represents the twin arginine translocation (RR) signal and T2, T3 are sites where copper binds.

EPI24 identified the responsible genes for the laccase activity. Multiple alignment of protein sequences of bacterial laccases showed that these enzymes are highly conserved. This indicates a huge importance in different mechanisms related to pigment production, morphogenesis, toxin oxidation, metals homeostasis, detoxification of xenobiotics and tolerance to oxidizing agents and UV light [4,42]. Our genome sequencing effort shows that the epiphytic bacteria *A. denitrificans* strain EPI24, isolated from the macroalgae *U. lactuca,* is a promissory source of lacasses with a potential application in bioremediation of phenolic compounds.

3. Nucleotide sequence accession number

The genome sequence of *Achromobacter denitrificans* strain EPI24 was submitted at DDBJ/ENA/GenBank under accession number PRJEB56270. EPI24 is available in the Bank of Strains and Genes of the Instituto de Biotecnología de la Universidad Nacional de Colombia under strain code IBUN-04284.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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