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Complete genome sequence of *Dyadobacter* sp. 32, isolated from a culture of the freshwater diatom *Cymbella microcephala*

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Abstract

Bacteria have been shown to be involved in different species-specific interactions with eukaryotic algae such as diatoms, impacting important ecosystem processes. Recently, a strain assigned to *Dyadobacter*, named 'species 32', has been shown to be involved in a number of ecologically relevant diatom processes, such as biofilm formation or growth enhancement, depending on the diatom species. This bacterium was originally isolated from a culture of freshwater benthic diatoms that originated from an epilithic biofilm, in which both bacteria and diatoms coexist. A single complete circular chromosome of *Dyadobacter* sp. 32 was assembled with a length of 7,101,228 bp, containing 6062 protein coding genes and 3 rRNA operons. A number of interesting genetic features were found, such as a putative zeaxanthin biosynthetic gene cluster. A large number of polysaccharide utilizing gene clusters were also detected, along with genes potentially acquired from other bacteria through horizontal gene transfer, and genes previously identified

in other algae-bacteria interactions. These data serve to increase our understanding of specific interactions within freshwater biofilms, and identify a number of gene targets with which to study the molecular basis of diatom-bacteria interactions.

Keywords: biofilm; zeaxanthin; interspecies signaling; phycosphere; extracellular polymeric substances

1. Introduction

In aquatic environments, diatoms are closely associated with bacteria, and the interactions between these microbes have global consequences (Seymour et al., 2017). Certain species of bacteria and diatoms readily colonize the benthos, where they are often the most abundant taxa (Van Colen et al., 2014). In doing so, they form biofilms: diverse communities of microorganisms, living in close quarters, connected by a complex interconnected material known as extracellular polymeric substances (EPS), consisting of mostly carbohydrates. These biofilms and the microbes within play a myriad of ecological roles, ranging from fostering the attachment of multicellular organisms (Ganesan et al., 2010), to stabilizing the sediment upon which the biofilm has formed (Chen et al., 2017). When biofilms form on man-made structures they can have detrimental consequences, as this so-called biofouling leads to the decay of machinery, and in the case of ships' hulls, increased drag, leading to greater fuel costs (Schultz et al., 2011). Hence, the characterization of the bacteria involved in biofilm formation is an important step in understanding (and potentially preventing) biofilm formation.

In freshwater environments, biofilms are not only industrially and ecologically important, but here they also live at the nexus of the overlying water body, groundwater, upstream environments and land runoff, while also influencing habitats downstream (reviewed in Battin et al. (2016)). Notably, research shows the biology of benthic freshwater diatoms is dictated by the associated cohort of bacteria. One strain, *Dyadobacter* sp. 32, originally isolated with the freshwater diatom *Cymbella microcephala*, itself isolated from biofilms of Lake Constance, was shown to have significant impacts on diatom biofilm formation, and

metabolite secretion. For example, certain benthic freshwater diatoms excrete more soluble carbohydrate when co-cultured with *Dyadobacter* sp. 32 (Bruckner et al., 2011), in some cases more than ten-fold in comparison to axenic diatom cultures. The authors also observed changes in the dissolved free amino acid content of these diatom-bacteria co-cultures, which involved both increases and decreases in the content of different amino acids. Further research focusing on the diatom *Achnanthidium minutissimum* showed biofilm formation and EPS secretion to be absent in axenic cultures. However, when this diatom was co-cultured with *Dyadobacter* sp. 32, the diatom adhered to the substratum, forming a biofilm and secreting a large "capsule" of EPS surrounding each cell (Windler et al., 2015). This was achieved even when the diatom was treated with sterile spent media, or solid phase extracts from *Dyadobacter* sp. 32 monocultures. This research showed that this bacterium (and its metabolites) can dictate metabolite secretion and biofilm formation in diatoms. Therefore, the genome and metabolic potential of the bacterium *Dyadobacter* sp. 32 is of particular interest in understanding diatom-bacteria interactions, diatom EPS secretion and biofilm formation.

Originally proposed by Chelius and Triplett with the type species *D. fermentans* (Chelius and Triplett, 2000), the genus *Dyadobacter* comprises at least 14 fully characterized species. These species are Gram negative staining, rod like, non-motile and aerobic, contain a flexirubin like pigment, and typically possess GC contents of around 48 %. They have generally been isolated from soil samples, as well as freshwater environments, and in association with vascular plants (Reddy and Garcia-Pichel, 2005). This article describes the genome of the isolate *Dyadobacter* sp. 32, isolated from benthic biofilms of Lake Constance. The level of 16S rRNA gene sequence similarity of strain 32 with other Dyadobacter species is between 92.65 and 94.29 %, based on the EZTaxon type strain database (Yoon et al., 2017).

2. Data description

Dyadobacter sp. 32 was grown using half-strength LB media under aerobic conditions at 20 °C with shaking (70 rpm), and DNA extracted using the CTAB method described by the Joint Genomes Institute (Feil et al., 2012). A second DNA sample was extracted from colonies grown on R2A media, using the Maxwell RSC instrument (Promega). Genome sequencing was conducted using Illumina MiSeq v3 (using a TruSeq nano kit for library prep) and PacBio RSII sequencing platforms (Pacific Biosystems), achieving coverage of 209× and 197×, respectively. Genome assembly was achieved with reads from both sequencing technologies, using the hybrid assembly method (at 'normal' stringency settings) of Unicycler (Wick et al., 2017). The assembled genome was annotated using the integrated microbial genomes expert review annotation pipeline version v. 5.0.2 (Markowitz et al., 2012).

The general genome and physiological characteristics of *Dyadobacter* sp. 32 are summarized in Table 1. The completed genome has a size of 7,101,228 bp, organized in a single chromosome. Annotation identified 6123 genes, 6062 of which are protein coding. The genome contains three ribosomal RNA operons, one of which contains two tRNA genes (isoleucine and alanine, respectively) between the 16S 23S genes (Figure 1). The sudden 'flip' in GC skew values was not aligned with the locus of the chromosomal replication initiator protein DnaA (locus tag DYADSP32_1). Instead, these two features were separated by roughly 1 mbp, much like the genome sequence of *D. fermentans* (Lang et al., 2009).

The genome was surveyed for secondary metabolite biosynthetic gene clusters, using the antiSMASH online tool v. 5.0.0 (Blin et al., 2019). Five biosynthetic gene clusters were detected, the results of which are summarized in SI 1. The role of one such gene cluster (Region 4), was putatively assigned as carotenoid biosynthesis. The gene cluster contains one regulatory element and five biosynthesis related genes, including a beta-carotene 3-hydroxylase (EC 1.14.15.24), utilized in the biosynthesis of zeaxanthin (see Figure 3). This gene cluster was identified in every other *Dyadobacter* strain, except for *D. ginsengisoli*

DSM 21015 (SI 1). In order to identify the pigment synthesized, *D. ginsengisoli* was acquired from the DSMZ, and the pigments of both strains were extracted following the pigment extraction and HPLC analysis by Lepetit et al (2013). This analysis identified a pigment from *Dyadobacter* sp. 32, which matched the retention time and absorption spectrum of a known zeaxanthin standard, and was not present in extracts of *D. ginsengisoli* (SI 2). These data suggest this gene cluster synthesizes zeaxanthin, or a pigment very similar to it.

The ability to process a wide range of carbohydrates is a common property of the Bacteroidetes phylum, and indeed novel carbohydrate utilizing enzymes have been identified in other species of *Dyadobacter* (Nihira et al., 2015). Given *Dyadobacter* sp. 32 is capable of growth when only supplied by carbohydrates secreted by diatoms, its genome could shed light on the methods by which bacteria utilize the unique carbohydrates of diatoms. To this end, the genome was surveyed for carbohydrate gene clusters using the dbCAN2 meta server (Zhang et al., 2018). This analysis detected 97 putative gene clusters, shown in SI 3. This value is within the same range of polysaccharide utilizing loci found in other *Dyadobacter* species by Terrapon and coworkers (2018).

Finally, the genome was scanned for genomic islands, using IslandViewer 4 (Bertelli et al., 2017), shown in SI 4. This analysis detected 24 genomic islands using at least one detection method, the largest of which being a 104 kbp island containing 114 genes. In fact, when homologs of each gene from this region were searched for using a standard BLAST search, one gene (locus tag DYADSP32_1929) was most similar to a protein encoded by *Kordia algicida* (NCBI gene accession code WP_040559848.1), a bacterium which excretes diatom-lethal proteases (Paul and Pohnert, 2011; Sohn et al., 2004). In neither bacterium is this gene a protease, nor does *Dyadobacter* sp. 32 appear to be detrimental to diatom growth. However, it is tempting to hypothesize that this putatively horizontally transferred gene confers an advantage during interactions with diatoms. Intrigued by this discovery, the genome was examined further for genes identified in other algae-bacteria interactions. Through this search it was found that *Dyadobacter* sp. 32

also harbors the so-called Ebo operon (DYADSP32_2563-DYADSP32_2568), a six gene operon identified in the genomes of both eustigmatophyte algae and bacteria, as illustrated by Yurchenko and coworkers (Yurchenko et al., 2016). While this operon has been hypothesized as synthesizing a novel metabolite, it is not clear if the operon has a function in algae-bacteria interactions.

The whole genome sequence of *Dyadobacter* sp. 32 is a valuable tool for understanding inter-species signaling in freshwater biofilms, which contributes to biofouling. The genomic analyses outlined above have identified a potentially novel zeaxanthin biosynthetic gene cluster. Furthermore, these analyses outline a number of targets for future studies regarding the ecological roles this bacterium plays, including genes which may help facilitate diatom-bacteria interactions.

3. Genome Accessions

The associated data of this publication are available under the bioproject accession PRJEB33118. The annotated genome is available under the accession code LR732074, and the strain has been deposited in the Belgian Coordinated Collection of Microorganisms under the accession code LMG 31449.

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Conflict of interest

The authors declare no conflict of interest.

Table 1: general features of *Dyadobacter* sp. 32

Property	Description

General features	
Classification	Domain Bacteria
	Phylum Bacteroidetes
	Class Cytophagia
	Order Cytophagales
	Family Cytophagaceae
	Genus Dyadobacter
Gram stain	Negative
Cell shape	Rods
Colony color	Yellow
Motility	Non-motile
Temperature range	8 – 30 °C
NaCl range	0 – 5 % (w/v)
Oxygen requirements	Aerobic
MIxS data	
BioProject accession	PRJEB33118
Investigation type	bacteria_archaea
Latitude and longitude	47.695396, 9.193617
Depth	0 m
Geographic location	Germany
Environment (biome)	Freshwater environment (ENVO:01000306)
Environment (feature)	Freshwater lake (ENVO:00000021)
Environment (material)	Biofilm (ENVO:00002034)
Elevation	395 m

Trophic level	Heterotroph

Sequence features

Sequencing platforms	Illumina MiSeq, PacBio RS
Assembly method	Unicycler v. 0.4.4
Size (bp)	7101228
GC content (%)	44.93
Genes	6123
Protein coding genes	6062
rRNA operons	3
tRNA genes	40
Replicons	1

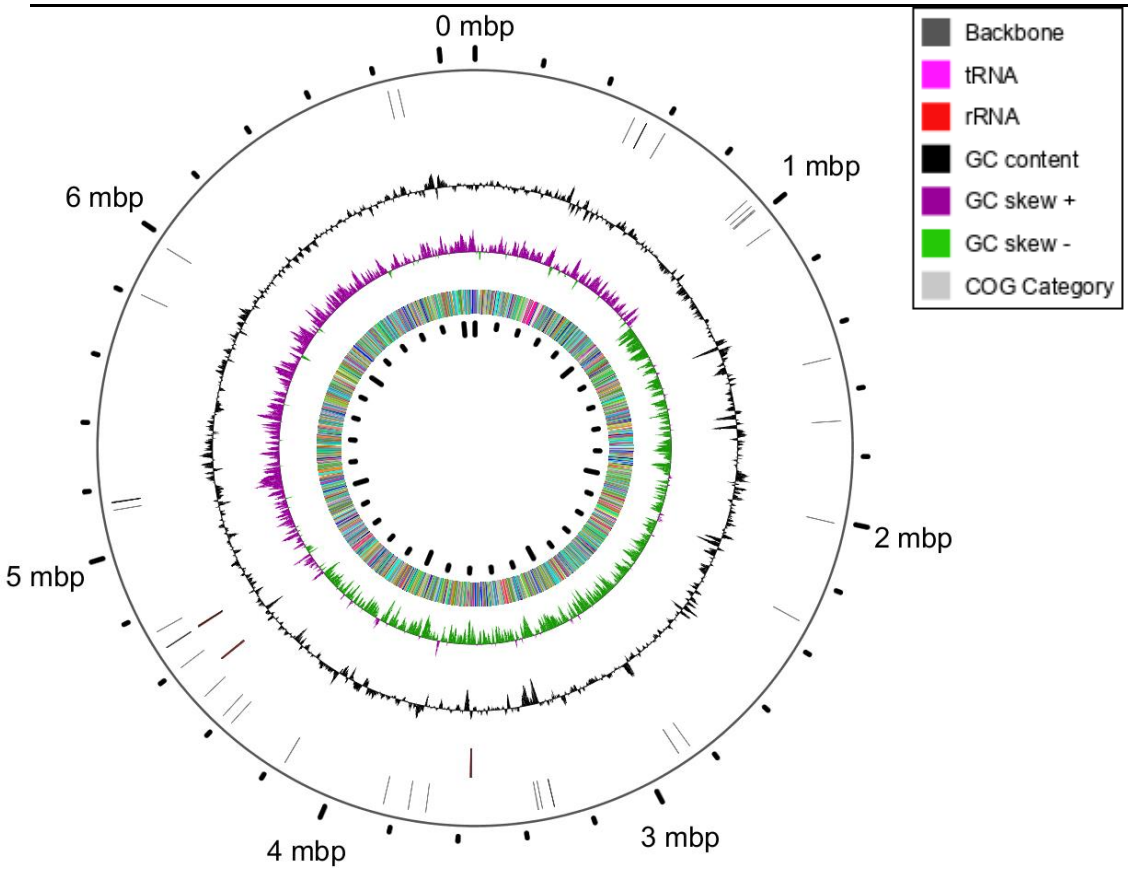

Figure 1: Complete genome of *Dyadobacter* sp. 32, produced with GView v. 1.7 (Petkau et al., 2010). The rings illustrate the following features, from the outside in: Gene coordinate (in mbp); tRNA genes; rRNA genes; GC content; GC skew; and COG category.

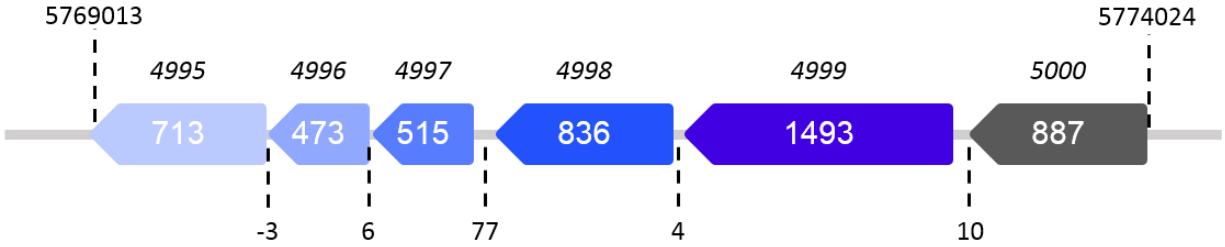

Figure 2: Putative zeaxanthin biosynthetic gene cluster structure. Six genes were identified, including one regulatory function (gray), and five biosynthetic elements (blue hues). The locus coordinates are shown for the beginning and end of the gene cluster above, along with the locus tag for each gene, with the locus tag prefix omitted (*4995-5000*). The length of each gene (in bp) is shown within the gene symbol, and distance between each gene is shown below (*4995* and *4996* overlap by 3 bp). *5000*: MerR DNA-binding transcriptional regulator; *4999*: Phytoene desaturase; *4998*: Phytoene/squalene synthetase; *4997*: Isopentenyl-diphosphate delta-isomerase; *4996*: Beta-carotene 3-hydroxylase; *4995*: Lycopene cyclase domain-containing protein.

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