Draft genome of *Elstera litoralis*, a freshwater epilithic biofilm associated bacterium from littoral zone of Lake Constance

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A B S T R A C T

Elstera litoralis, is a Rhodospirillaceae member which was isolated from the littoral zone of Lake Constance from a stone biofilm using diatom extracellular polymeric substances (EPS) as C source. We present here the draft genome of *E. litoralis* which has a genome size of 3.83 Mb and 61.2% G + C content. Genome analysis indicated utilization of multiple C substrates explaining its heterotrophic lifestyle as a bacterium present in natural biofilms. Further comparative genome analysis of *Elstera* with other members of Rhodospirillaceae would be helpful to understand the evolutionary relationships and divergence of hydrobacteria from terrabacteria.

Keywords:

Elstera litoralis
Epilithic biofilms
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Draft genome

Elstera is a recently described genus within the family Rhodospirillaceae, of alpha proteobacteria with a single species, *Elstera litoralis* (Rahalkar et al., 2012). When isolated, it was most related to *Azospirillum* spp. (~90–92%) based on its 16S rRNA gene sequence, however after the description of a lake bacterium, *Lacibacterium aquatile* (Sheu et al., 2013), *E. litoralis* was found to be most related to *L. aquatile* on the basis of 16S rRNA gene by 94% similarity. *E. litoralis* was isolated from epilithic biofilms from the littoral zone of Lake Constance (Rahalkar et al., 2012). Lake Constance is the second largest freshwater lake in Europe and is characterized by a littoral zone comprising of stones covered with biofilms. In our previous studies, we have shown that diatom EPS (Bahulikar and Kroth, 2008) can act as C source for heterotrophic bacteria (Bruckner et al., 2008). *E. litoralis* was isolated as one of the most dominant heterotroph from such a biofilm using diatom EPS as the C source in a following study (Rahalkar et al., 2012). Diatoms and bacteria are the dominant members of the biofilm community in the littoral zone of Lake Constance where EPS produced by diatoms can be used by heterotrophic organisms as a carbon source (Bruckner et al., 2008). To the best of our knowledge, there are no reported complete or draft genome sequences of organisms isolated from freshwater epilithic biofilms. Here we announce the draft

genome of *E. litoralis* Dia 1^T. The whole genome helped us in understanding the evolution and life strategies of this organism which is present naturally in complex environment such as a stone biofilm, occurring between land and water.

The genome of *E. litoralis* was sequenced using the Ion Torrent PGM (200 bp) with the 316™ sequencing chip using the manufacturer's instructions (Life Technologies, USA). De novo assembly was performed using the MIRA 4.0.5 assembler (Chevreux et al., 1999) which generated 1012 contigs, with N50 of 8551 bp and N90 of 2026 bp. This data was then submitted to the Rapid Annotation using Subsystem Technology (RAST server) (Aziz et al., 2008; Overbeek et al., 2014) and NCBI genome sequence database. RAST server was used for subsystem analysis where the subsystem coverage in this case was 46% belonging to 408 subsystems. Closest genome match of the genome was with *Rhodospirillum centenum* (genome ID: 414684.4) with 539 score, an aquatic bacterium in the same family. It was revealed that genome size of *E. litoralis* was 3,830,27 Mb with a G + C content of 61.2%. Annotation by NCBI prokaryotic genome annotation pipeline (PGAAP) http://www.ncbi.nlm.nih.gov/genome/annotation_prok/ revealed the presence of 1012 contigs with 3310 coding DNA sequences, 57 tRNAs and 14 rRNA (5S, 16S and 23S) with a single rRNA operon (Table 1).

The absence of any photosynthetic apparatus and genes of Calvin cycle was consistent with that of *Azospirillum* species in contrary to most of the other Rhodospirillaceae members (Wisniewski Dye et al., 2011; Baldani et al., 2014). As *E. litoralis* dwells in stone biofilms, primary production may not be necessary function, as these biofilms are mainly dominated by diatoms and algae that may provide organic carbon for heterotrophic community members. Thus, *E. litoralis* may be

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Table 1
Genome features of *Elstera litoralis*.

Attributes	Values
Genome size	3.83 Mb
Contigs	1012
G + C content	61.2%
CDs	3310
tRNA	57
rRNA	14 (5S, 16S, 23S)
ncRNA	1

adapted to a heterotrophic metabolism, in contrast to the autotrophic lifestyle observed in other members of the family. This was reflected in the detection of genes involved in the utilization of variety of substrates, including sugars, amino sugars, sugar alcohols, etc. as previously observed experimentally (Rahalkar et al., 2012). A capacity for utilizing various monosaccharides such as mannose, fucose, ribose, arabinose, rhamnose, xylose, and fructose was also observed. The extracellular polysaccharides of benthic diatoms are commonly composed of rhamnose, fucose, xylose, mannose, galactose, glucose, and other monomers (Bahulikar and Kroth, 2008). These sugar monomers are also found in natural biofilms dominated by diatoms (Bahulikar, 2006). The genome also exhibited genes for utilization of N acetyl glucosamine, chitin, myo inositol, putrescine, ethanolamine, glycerol, alginate which are amongst the most important common naturally occurring carbon substrates in the sticky biofilm environments (Sutherland, 2001). The chemotaxis genes and flagellar apparatus were detected which are particularly important in the biofilm environment for movement towards C sources.

Azospirillum is the next phylogenetic relative of *Elstera* after *Lacibacterium*, as revealed in NCBI blast. Genomes of *Azospirillum* species have been studied for their evolutionary origin (Wisniewski Dye et al., 2012) as they are the only terrestrial organisms amongst the Rhodospirillaceae family. *Azospirillum* is one of the most well studied organisms for its association with important cereals and grasses. In a detailed comparative genome analysis of *Azospirillum* spp. it was suggested that it might have diverged much later than the suggested Precambrian split of 'hydrobacteria' to 'terrabacteria'. Also, the separation of *Azospirillum* from their close aquatic relatives coincided with the emergence of vascular plants on land (Wisniewski Dye et al., 2011). Bacteria associated with stone biofilms such as *E. litoralis*, can also be also explored as a possible link for the transition from aquatic to terrestrial habitats, as they are present physically on the borderline connecting land and water. Thus, detailed study of *E. litoralis* genome can further shed light on the evolutionary divergence of 'hydrobacteria' and 'terrabacteria'. *Azospirillum* genome has acquired nearly half of its genes by horizontal transfer from terrestrial organisms like *Burkholderia* species. In preliminary analyses, we checked the origin for some of the genes in *E. litoralis*. We observed some of the genes showing ancestral

origin, e.g. genes involved in the TCA cycle, *nirB*, flagella, DNA gyrase, etc. In contrast a few of the other genes, involved in the utilization of sugars, etc. showed similarity to *Burkholderia*, *Rhizobium* etc. indicating horizontal gene transfer (details not shown). A detailed study comparing the *Elstera* genome with genomes of other members of the family Rhodospirillaceae including *Azospirillum* will help us to better understand these interesting aspects of microbial evolution.

Nucleotide sequence accession number

The draft genome sequence of *E. litoralis* was deposited in the DDBJ/EMBL/GenBank database under accession number LAJY00000000.1 (version 01).

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