First Complete Genome Sequences of *Janthinobacterium lividum* EIF1 and EIF2 and Their Comparative Genome Analysis

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Abstract

We present the first two complete genomes of the *Janthinobacterium lividum* species, namely strains EIF1 and EIF2, which both possess the ability to synthesize violacein. The violet pigment violacein is a secondary metabolite with antibacterial, antifungal, antiviral, and antitumoral properties. Both strains were isolated from environmental oligotrophic water ponds in Göttingen. The strains were phylogenetically classified by average nucleotide identity (ANI) analysis and showed a species assignment to *J. lividum* with 97.72% (EIF1) and 97.66% (EIF2) identity. These are the first complete genome sequences of strains belonging to the species *J. lividum*. The genome of strain EIF1 consists of one circular chromosome (6,373,589 bp) with a GC-content of 61.98%. The genome contains 5,551 coding sequences, 122 rRNAs, 93 tRNAs, and 1 tm-RNA. The genome of EIF2 comprises one circular chromosome (6,399,352 bp) with a GC-content of 61.63% and a circular plasmid p356839 (356,839 bp) with a GC-content of 57.21%. The chromosome encodes 5,691 coding sequences, 122 rRNAs, 93 tRNAs, and 1 tm-RNA and the plasmid harbors 245 coding sequences. In addition to the highly conserved chromosomally encoded violacein operon, the plasmid comprises a non-ribosomal peptide synthetase cluster with similarity to xenoamicin, which is a bioactive compound effective against protozoan parasites.

Key words: *Janthinobacterium lividum*, secondary metabolites, violacein operon, antimicrobial, antifungal, xenoamicin.

Introduction

Janthinobacterium lividum is a betaproteobacterium and belongs to the family of *Oxalobacteraceae*. This family comprises 13 genera including the genus *Janthinobacterium* (Baldani et al. 2014), which in turn contains the species

J. lividum (Kämpfer et al. 2008), *Janthinobacterium svalbardensis* (Ambrozic Avgustin et al. 2013), and *Janthinobacterium agaricidamnosum* (Lincoln et al. 1999) as well as the recently announced species *Janthinobacterium violaceinigrum* sp. nov., *Janthinobacterium aquaticum* sp.

Significance

The species *Janthinobacterium lividum* is known for producing a variety of secondary metabolites. Those bioactive compounds are valuable for different biotechnological applications. Comparative genomics of *J. lividum* investigating the overall genomic structure and genome evolution are limited due to the lack of complete genome sequences. The here analyzed new isolates and their complete genomes give insight into their potential for bioactive compound discovery including violacein and xenoamicin. Moreover, we show that the chromosomes of *J. lividum* EIF1 and EIF2 have a conserved genome structure and that these two novel strains function as a blueprint for future genome comparisons throughout the *Janthinobacterium* genus, which deepens our understanding of this genera's evolution.

nov., and *Janthinobacterium rivuli* sp. nov. (Huibin et al. 2020). Members of *Janthinobacterium* are Gram-negative, motile, and rod-shaped (Baldani et al. 2014). They are strictly aerobic, chemoorganotrophic, and are proposed to grow at a temperature optimum of 25–30 °C (Baldani et al. 2014). In addition, psychrophilic isolates are known that are able to grow at 4 °C (Suman et al. 2015). *Janthinobacterium* strains inhabit different environments including soil (Asencio et al. 2014; Shoemaker et al. 2015; Wu et al. 2017), various aquatic habitats such as lakes (Suman et al. 2015), water sediments (McTaggart et al. 2015), and rainwater cisterns (Haack et al. 2016). Some *Janthinobacterium* isolates are also known as beneficial skin microsymbionts of amphibians (Brucker et al. 2008; Harris et al. 2009) and as pathogens of rainbow trouts (Oh et al. 2019). *Janthinobacterium* colonies have a purple-violet color produced by the pigment violacein. This colorful secondary metabolite (SM) is known to exhibit antimicrobial, antiviral, and antitumor properties (Andrighetti-Fröhner et al. 2003; Bromberg et al. 2010; Asencio et al. 2014) and thus bears great potential for biotechnological applications. In the current study, we assessed 1) the first complete genomes of two novel *J. lividum* isolates EIF1 and EIF2 and compared 2) the genetic localization of the violacein cluster within the genus *Janthinobacterium*. Additionally, we focused on 3) the potential for synthesis of bioactive SMs.

Materials and Methods

Isolation, Growth Conditions, and Genomic DNA Extraction

Janthinobacterium lividum EIF1 and EIF2 were obtained from environmental oligotrophic water surface and plant material including leaves and stem from opposite-leaved pondweed, *Groenlandia densa*. The samples were collected in Göttingen (Germany) on 11.09.2018 (51°33′58″N 9°56′22″E). Enrichment cultures were performed by using environmental water samples to inoculate peptone medium containing 0.001% (w/v) peptone (Carl Roth GmbH + Co. KG, Karlsruhe, Germany). Cultures were allowed to stand undisturbed for 3 weeks at 25 °C (Poindexter 2006). Both biofilm and water surface material were sampled and streaked on 0.05% peptone-containing agar medium supplemented with 1% vitamin solution No. 6 (Staley 1968). After colony formation, they were transferred to a new agar plate containing diluted peptone medium supplemented with CaCl$_2$ (PCa) (Poindexter 2006) and incubated for 4 days at 25 °C. For singularization of isolates, restreaking was performed at least four times. Individual single colonies were cultured in liquid PCa medium and genomic DNA was extracted with the MasterPure complete DNA and RNA purification kit as recommended by the manufacturer (Epicentre, Madison, WI). After the addition of 500 µl Tissue and Cell Lysis Solution, the resuspended cells were transferred into Lysing Matrix B tubes (MP

Biomedicals, Eschwege, Germany) and mechanically disrupted for 10 s at 6.5 m/s using FastPrep-24 (MP Biomedicals). The supernatant was cleared by centrifugation for 10 min at 11,000 × g, transferred into a 2.0-ml tube and 1 µl Proteinase K (20 mg/ml) (Epicentre) was added. The procedure was performed as recommended but the MPC Protein Precipitation Reagent was modified to 300 µl. The 16S rRNA genes of purified isolates were amplified with the primer pair 27F and 1492R (Fredriksson et al. 2013). Sanger sequencing of the polymerase chain reaction products was done by Seqlab (Göttingen, Germany).

Genome Sequencing, Assembly, and Annotation

Illumina paired-end sequencing libraries were prepared using the Nextera XT DNA Sample Preparation kit and sequenced by employing the MiSeq-system and reagent kit version 3 (2× 300 bp) as recommended by the manufacturer (Illumina, San Diego, CA). For Nanopore sequencing, 1.5 µg DNA was used for library preparation employing the Ligation Sequencing kit 1D (SQK-LSK109) and the Native Barcode Expansion kit EXP-NBD103 (Barcode 3) for strain EIF1 and the Native Barcode Expansion kit EXP-NBD104 (Barcodes 7 and 12) for strain EIF2 as recommended by the manufacturer (Oxford Nanopore Technologies, Oxford, UK). Sequencing was performed for 72 h using the MinION device Mk1B and a SpotON Flow Cell R9.4.1 as recommended by the manufacturer (Oxford Nanopore Technologies) using MinKNOW software v19.05.0 for sequencing (strain EIF1 and first run of EIF2) and v19.06.8 for the second run of strain EIF2. For demultiplexing, Guppy versions v3.0.3 (strain EIF1), v3.1.5 (strain EIF2, first run), and v.3.2.1 (EIF2, second run) were used. Illumina raw reads were quality filtered with fastp v0.20.0 (Chen et al. 2018) using the following parameters: base correction by overlap, base phred score ≥Q20, read clipping by quality score in front and tail with a sliding window size of 4, a mean quality of ≥20, and a required minimum length of 50 bp. Reads were additionally adapter trimmed by using cutadapt v2.5 (Martin 2011). For adapter trimming of Oxford Nanopore reads, Porechop (https://github.com/rrwick/Porechop.git; last accessed April 29, 2019) was used with default parameters. Quality filtering with fastp v0.20.0 (Chen et al. 2018) was performed by using following parameters: base phred score ≥Q10, read clipping by quality score in front and tail with a sliding window size of 10, a mean quality of ≥10, and a required minimum length of 1,000 bp.

Janthinobacterium lividum EIF1 was de novo assembled using Unicycler v0.4.8 in normal mode (Wick et al. 2017) and quality checked with Bandage v0.8.1 (Wick et al. 2015).

For *J. lividum* EIF2, a de novo long-read-only assembly with Nanopore reads was performed using Unicycler v0.4.8 due to repetitive transposases in the genome and to avoid misassemblies of overrepresented repetitive regions by short-reads. To increase the quality of the Nanopore assembly, additional

polishing was performed with unicycler-polish (https://github.com/rrwick/Unicycler/blob/master/docs/unicycler-polish.md; last accessed February 13, 2020) by mapping Illumina short-reads with bowtie2 v2.3.5.1 (Langmead and Salzberg 2012) against the Nanopore-based assembly and base correction by Pilon 1.23 (Walker et al. 2014). This routine corrects substitutions, indels as well as larger variants such as repetitive homo-stretches, deletions, and large deletions. The contiguity of the assembly was manually inspected and evaluated with Tablet v1.19.09.03 (Milne et al. 2013). Quality of the assembled genomes was assessed with CheckM v1.1.2 (Parks et al. 2015) and genome annotation was performed by using the Prokaryotic Genome Annotation Pipeline v4.11 (Tatusova et al. 2016) and subsequent manual curation of the genes encoding the violacein operon.

Phylogenetic Classification

The Genome Taxonomy Database Toolkit (GTDB-Tk) v1.0.1 (Chaumeil et al. 2019) was used to provide an initial taxonomic placement of the *J. lividum* isolates. Subsequently, an in-depth phylogenetic analysis was performed using ANIm method of pyani (https://github.com/widdowquinn/pyani v0.2.9; last accessed March 05, 2020) (Arahal 2014) as described by Richter and Rosselló-Móra (2009). Based on the list of the type strain collection of the German Strain Collection of Microorganisms and Cell Cultures GmbH (DSMZ, Braunschweig, Germany), available type strain genomes were downloaded from the National Centre for Biotechnological Information (NCBI, accessed 05.03.2020) including *J. lividum* NCTC9796[T] (PRJEB6403), *J lividum* H-24[T] (PRJNA309034), *J. svalbardensis* strain PAMC 27463[R] (PRJNA407061), and *J. agaricidamnosum* DSM 9628[T] (PRJEB4003). The type strain genome of the species *J. lividum* was sequenced twice (NCTC9796[T] and H-24[T]). Both were included in the analysis due to their incomplete sequencing status, difference in coverage, and used annotation pipelines.

Comparative Genomics

To investigate the metabolic potential of *J. lividum* EIF1 and EIF2, BlastKOALA v2.2 (Kanehisa et al. 2016) was used. Putative SM biosynthetic gene clusters and putative phage regions were identified with AntiSMASH v5.1.2 (Blin et al. 2019) and PHASTER (Arndt et al. 2016), respectively. Comparative analysis and visualization of the violacein operon was performed with Easyfig v2.2.3 (Sullivan et al. 2011). Whole-genome comparisons were performed by employing the BLAST Ring Image Generator v0.95 (Alikhan et al. 2011).

Results and Discussion

Genomic Features of *J. lividum* EIF1 and EIF2

We present the first complete genomes of two *J. lividum* strains EIF1 and EIF2, which originate from a surface water sample and a pondweed plant in Göttingen, respectively. The sequencing statistics are summarized in supplementary table S1, Supplementary Material online. The complete genomes were assembled from quality-filtered Oxford Nanopore reads (EIF1: 41,395 and EIF2: 237,547) with a mean length of 8,045 bp (EIF1) and 5,954 bp (EIF2) and Illumina reads with 3,271,600 (EIF1) and 2,562,634 (EIF2) reads in total. The de novo hybrid genome assembly of *J. lividum* EIF1 yielded a 6,373,589-bp circular chromosome, with a GC-content of 61.98% and a coverage of 181.3-fold. Short-read polished long-read Nanopore assembly of *J. lividum* EIF2 resulted in a circular chromosome (6,399,352 bp) and a circular plasmid (356,839 bp) with a coverage of 298.9-fold and 343.6-fold and a GC-content of 61.63% and 57.21%, respectively. In total, short-read polishing corrected 321 variants including substitutions, insertions, homo-stretches, deletions, and large deletions. Both assemblies were evaluated manually with Bandage v0.8.1 (Wick et al. 2015) and Tablet 1.19.09.03 (Milne et al. 2013). No CRISPR regions were detected in both genomes.

Phylogeny of *J. lividum* EIF1 and EIF2

The quality of the assemblies was evaluated with CheckM v1.1.2 (Parks et al. 2015) and revealed high purity with a completeness of 99.6% and a contamination rate of 2.38% (EIF1) and 1.58% (EIF2), respectively. The first taxonomic assignment of GTDB-Tk v1.0.1 (Chaumeil et al. 2019) based on fastani values (97.66-EIF1 and 97.5-EIF2) demonstrated that both strains belong taxonomically to the species *J. lividum*. The available type and representative strains were used in the pyani analysis, which revealed that both *J. lividum* EIF1 and EIF2 build a cluster with the type strains *J. lividum* H-24[T] and NCTC 9796[T] (fig. 1A). In detail, *J. lividum* EIF1 and EIF2 cluster with 97.72% and 97.66% sequence identity, respectively, to the type strains *J. lividum* H-24[T] and NCTC 9796[T]. This is above the species boundary of ~94–95% and allows a reliable classification of both isolates EIF1 and EIF2 to the species *J. lividum*. The genomes of strains EIF1 and EIF2 share a sequence identity of 98.48%.

Metabolism and Production of Bioactive Compounds

Functional prediction of gene clusters was conducted with BlastKOALA. In total, 54.2% (EIF1) and 52.2% (EIF2) genes fall into 23 functional categories according to the Kyoto Encyclopedia of Genes and Genomes orthology. Among all categories, genes involved in signaling and processing (EIF1, 409 genes; EIF2, 431 genes), environmental processing (EIF1, 378 genes; EIF2, 391 genes), and genetic information
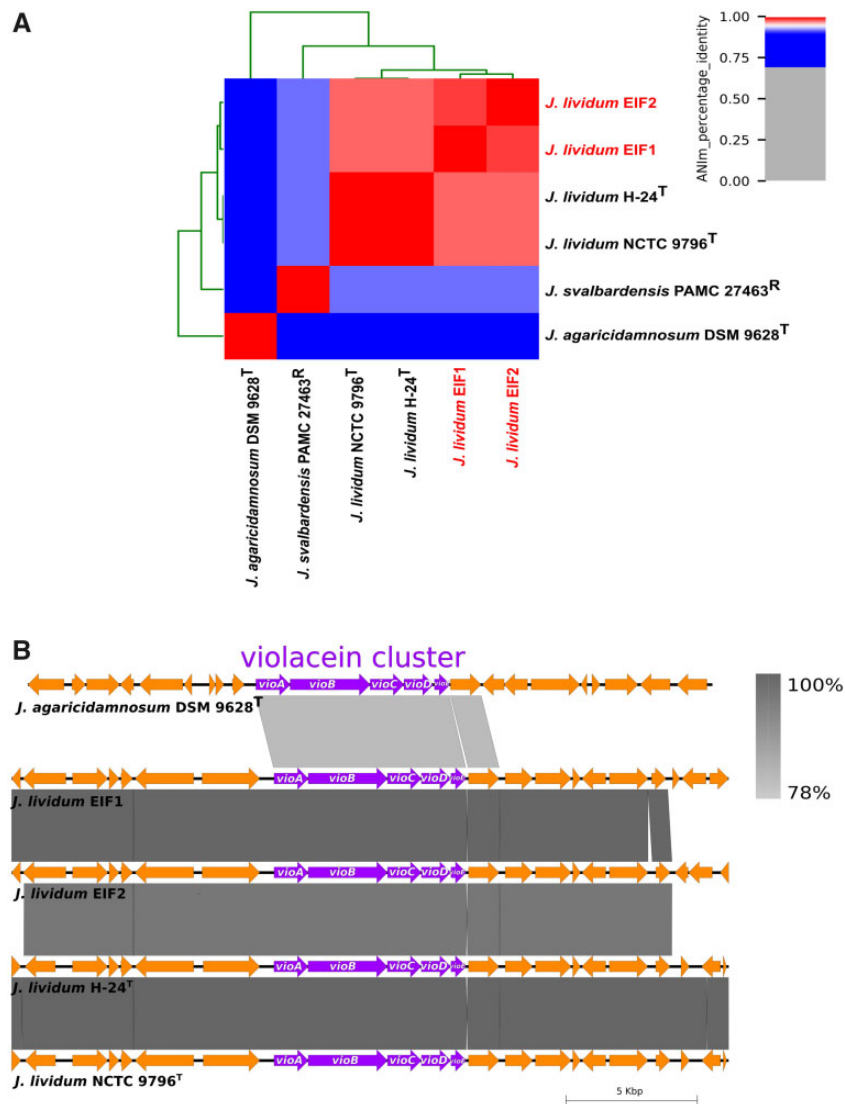
Fig. 1.—(*A*) Phylogenetic analysis of *Janthinobacterium lividum* EIF1 and EIF2 and (*B*) comparison of violacein operon of *Janthinobacterium*. (*A*) All available type strains ([T]) and representative strains ([R]) from the genus *Janthinobacterium* were considered. Calculations were done with pyani (https://github.com/widdowquinn/pyani v0.2.9; last accessed March 05, 2020) (Richter and Rosselló-Móra 2009; Arahal 2014) using the ANIm method with standard parameter. (*B*) The violacein operon is indicated in purple and surrounding genes in orange. Gray shading indicates regions of homology based on nucleotide level. Visualization was performed with Easyfig 2.2.3 (Sullivan et al. 2011).

processing (EIF1, 352 genes; EIF2, 361 genes) were most abundant. Additionally, several genes were affiliated to metabolic processing for substrate conversion including carbohydrate (EIF1, 292 genes; EIF2, 293 genes), nitrogen (19 genes), and sulfur (30 genes).

In total, 186 genes in EIF1 and 187 genes in EIF2 were associated with cell signaling including quorum sensing (52 genes), biofilm formation (EIF1, 77 genes; EIF2, 78 genes), and cell motility (57 genes). This indicates flexible genomes that enable the microorganism to sense and process diverse stimuli and substrates from the environment.

Members of the genus *Janthinobacterium* are a promising source for novel pharmaceutical compounds, as they bear the

potential to synthesize important SMs with exceptional antibacterial, antifungal, antiviral, and antiprotozoal properties (Brucker et al. 2008; Wang et al. 2012; Asencio et al. 2014; Suman et al. 2015; Durán et al. 2016). Both isolated strains showed a purple color during growth in liquid and solid media (supplementary fig. S1, Supplementary Material online), indicating the production of bioactive pigments. Genome analysis with AntiSMASH v5.1.2 (Blin et al. 2019) revealed that EIF1 comprises six and EIF2 seven putative SM gene clusters. In both genomes, genes typical for synthesis of terpene, bacteriocins, and violacein were detected.

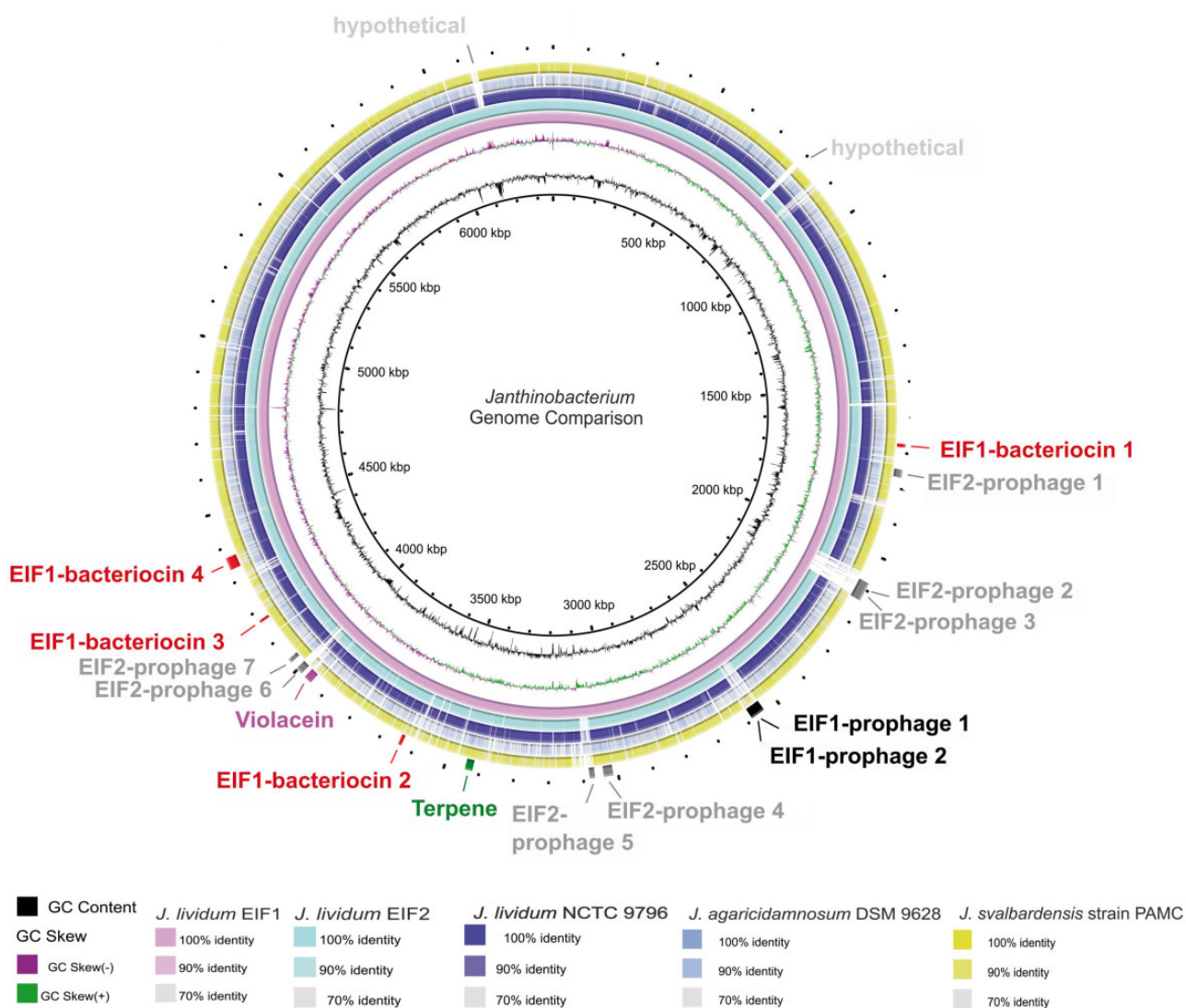The genomic comparison of the violacein operon (*vioABCDE* EIF1 3,947,675–3,970,695 bp and EIF2

FIG. 2.—Comparison of two new complete genomes of *Janthinobacterium lividum* strains EIF1 and EIF2 and three draft genomes of *J. lividum* NCTC 9796[T], *Janthinobacterium agaricidamnosum* DSM 9628[T], and *Janthinobacterium svalbardensis* strain PAMC 27463[R]. The figure was generated using BLAST Ring Image Generator (Alikhan et al. 2011). As central reference, the *J. lividum* EIF1 chromosome is depicted (black ring with size, GC-content, and GC skew are indicated). BLAST matches between *J. lividum* EIF1 and other strains are shown as concentric colored rings on a sliding scale according to percentage identity (100%, 90%, or 70%). Regions of differences are labeled: bacteriocins (red), terpene (green), violacein (pink), prophages EIF1 (black), prophages EIF2 (gray), and regions of differences with hypothetical function (light gray).

3,934,551–3,957,571 bp) and surrounding genomic regions revealed a high conservation of the entire operon in the genomes of all different *J. lividum* strains and other *Janthinobacterium* species, that is, *J. agaricidamnosum* (fig. 1B). The genomic surrounding indicates conservation at intraspecies level only. In addition, the *J. lividum* EIF2 plasmid p356839 encodes a putative nonribosomal peptide synthetase cluster. This cluster showed an overall similarity to other known clusters synthesizing xenoamicin A/xenoamicin B by comprising eight core biosynthetic genes (G8765_29435-G8765_29465, G8765_29475), five additional biosynthetic genes (G8765_29470, G8765_29490, G8765_29500, G8765_29505, and G8765_29540), and three transport-related genes (G8765_29520, G8765_29525, and G8765_29535). Xenoamicin A/B is known for its activity against *Plasmodium falciparum*, an unicellular protozoan parasite (Zhou et al. 2013). The comparison of the GC-content shows a difference of 4.42% between the chromosome and plasmid p356839 of strain EIF2 suggesting that the plasmid was obtained recently.

Comparative Genomics of *Janthinobacterium*

The two complete genomes of *J. lividum* (EIF1 and EIF2) presented here allow a reliable genomic structure simulation of incomplete available draft genomes of this species for the first

time. The overall genome comparison between *Janthinobacterium* genera revealed not only broad genome similarities but also species and strain specific differences (fig. 2) (Alikhan et al. 2011).

The chromosome organization of the species *J. lividum* follows a conserved structural genus blueprint, which is also detected in the species *J. agaricidamnosum* and *J. svalbardensis*. This highlights the high genomic conservation of the genus *Janthinobacterium*. To investigate regions of difference, the genomes were searched for putative prophage regions, which are known as drivers of genomic evolution (Casjens 2003; Brüssow et al. 2004; Canchaya et al. 2004). PHASTER analysis revealed two putative prophage regions (region 1: 2,543,591–2,585,344 bp; region 2: 2,565,749–2,585,385 bp) in *J. lividum* EIF1 (fig. 2). The regions comprise 41.7 and 19.6 kb and were classified as questionable and incomplete, respectively. However, both comprised phage attachment sites. In the genome of *J. lividum* EIF2, eight putative prophage regions were identified, of which seven reside within the chromosome (fig. 2) and one within the plasmid (315,441–324,647 bp, questionable). Two regions were classified as intact (region 1: 1,746,259–1,768,581 bp and region 3: 2,093,778–2,133,389 bp) and comprised the phage typical attachment sites AttL and AttR. These results support the hypothesis that bacterial strain diversification is mainly driven by phages interacting with the host chromosome (Canchaya et al. 2004) and extrachromosomal elements obtained by horizontal gene transfer (Giménez et al. 2019). Several SM clusters, such as the biosynthesis of bacteriocin, terpene, and violacein, were conserved among all investigated *J. lividum* isolates, indicating SM production as a common feature among this species.

In conclusion, we assembled two complete genomes derived from new isolates of the species *J. lividum* (EIF1 and EIF2) using Illumina and Nanopore technology. These are the first complete genomes described for this species and allowed in-depth genome analysis and comparisons. We have shown that both strains encode SM clusters, including the bioactive compounds violacein and xenoamicin A/B.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

Acknowledgments

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