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# Comprehensive genomic analysis of *Xenorhabdus bovienii* strain MEL2.2

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## Abstract

The genome sequences of entomopathogenic bacteria and their functional analyses provide valuable insights for genetic engineering to enhance their use as biocontrol agents. In this study, we examine the draft genome of *Xenorhabdus bovienii* strain MEL2.2, which was isolated from entomopathogenic nematodes in Melbourne, Australia. The genome of *Xenorhabdus* strain MEL2.2 spans approximately 4.4 million base pairs and has a G + C content of 44.8%, aligning with known characteristics of the genus. Within the genome, 3,823 protein-coding genes were identified. Functional analysis revealed genes associated with nematode symbiosis and insect virulence. Moreover, 15 biosynthetic gene clusters (BGCs) were detected, potentially responsible for synthesizing various secondary metabolites. Comparative genomic analysis indicated a combination of conserved and strain-specific genes when compared to other *Xenorhabdus bovienii* strains, suggesting genetic traits that may enhance MEL2.2's adaptability and pathogenicity. Altogether, these findings offer a foundation for exploring the strain's utility in further applications.

## Introduction

The genera *Xenorhabdus* and *Photorhabdus* include entomopathogenic bacteria associated with *Steinernema* and *Heterorhabditis* nematodes, respectively [1,2]. These bacteria participate in mutualistic symbiotic relationships with nematodes, facilitating the infection and killing of insect hosts [3]. Beyond their ecological role, *Xenorhabdus* and *Photorhabdus* species are prolific producers of secondary metabolites with significant biotechnological and pharmaceutical potential. These bioactive compounds, including non-ribosomal peptides, polyketides, and other bioactive compounds, are synthesized by specialized biosynthetic gene clusters (BGCs)

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encoded within their genomes [4]. The metabolic versatility of these bacteria makes them valuable candidates for genome-guided studies [5,6] aimed at discovering novel bioactive compounds.

In Australia, various *Xenorhabdus* and *Photorhabdus* species have been isolated from several environments, emphasizing their ecological and biotechnological importance. Species include *X. miraniensis*, known for producing antibiotics such as xenocoumacin and xenorhabdin [7], *X. beddingii* [8], and *X. bovienii* strain T228, which is remarkable for its entomopathogenic properties and bioactive compound production, making it a strong candidate for biological pest control [8]. In addition, some *Photorhabdus* species, including

*P. luminescens* [9] and *P. asymbiotica*, have been implicated in human infections, particularly skin lesions [10]. Recently, the novel *P. viridis* was isolated from *Heterorhabditis zealandica* Poinar, 1990 [11] nematodes in Brecon, Australia [12], further highlighting the growing interest in these bacteria as promising sources of novel bioactive metabolites.

This study reports the draft genome of *X. bovienii* strain MEL2.2, with particular emphasis on genes related to symbiotic relationships, pathogenic mechanisms, and the biosynthesis of secondary metabolites. This genomic characterization expands our understanding of *Xenorhabdus* diversity and contributes to the ongoing exploration of microbial natural products.

## Methods

### Isolation of entomopathogenic bacteria

The strain MEL 2.2 was isolated from Melbourne, Australia. First, free-living infective juveniles (IJs) of entomopathogenic nematodes (EPNs) were isolated from soil samples using the baiting technique [13], in which *Galleria mellonella* (Linnaeus, 1758) [14] larvae were placed in a plastic box with soil samples and left for 5 days. Dead larvae were collected for EPN isolation. The White trap technique was then used to isolate EPNs from deceased *G. mellonella* larvae by placing them on filter paper in a petri dish setup with sterile distilled water, followed by incubation in the dark at room temperature for 15–20 days. However, no emerged EPNs were observed, possibly due to failed nematode reproduction within the cadavers. Potential contributing factors include insufficient nutrient availability, or suboptimal temperature conditions, any of which could hinder infective juvenile (IJ) development or emergence [15,16]. Subsequently, symbiotic bacteria associated with EPNs were then extracted from the hemolymph of *G. mellonella* larvae infected with IJs. The larvae were surface-sterilized with 70% ethanol, air-dried, and dissected using sterile forceps to expose the hemolymph, which was streaked onto nutrient bromothymol blue-triphenyltetrazolium chloride agar (NBTA). Plates were incubated in the dark at room temperature for 48–72 hours, after which a single colony was cultured in Luria-Bertani (LB) broth for 24 hours. The resulting cell pellet was then suspended in LB broth supplemented with 20% glycerol and stored at −80°C for further studies.

### Genome sequencing, assembly and annotation

A single colony was selected for DNA extraction using the GenFind V2 kit (Beckman Coulter). Illumina library preparation and short-read sequencing were performed at Doherty Applied Microbial Genomics, The University of Melbourne, Melbourne, Australia. Library preparation was conducted using the Illumina DNA Prep kit with quarter reagent volumes, followed by short-read sequencing on an Illumina NextSeq 500 with a 150 bp paired-end kit. The raw data were assessed for quality-checked using Falco (Version 1.2.4) [17]. Reads were trimmed with Trimmomatic (Version 0.39) [18]. Genome assembly was carried out with SPAdes (Version 3.15.5) [19], employing the --cov-cutoff parameter. Genome annotation was performed using Prokka (Version 1.14.6) [20]. Taxonomic classification was then performed using 16S rDNA and *recA* sequence analysis, along with Kraken2 [21]. Finally, QUAST [22] was used to assess data quality.

### Genomic analyses

Gene function was analyzed using the EggNOG 5.0-mapper tool [23], which mapped the sequences to the EggNOG database and assigned functional categories such as Clusters of Orthologous Genes (COGs). Briefly, FASTA gene sequences from Prokka were submitted via the EggNOG web interface. The results were then visualized using Python to display the functional distribution. Analysis of BGCs in strain MEL2.2 was conducted using AntiSMASH version 7.0 [24]. Following automated predictions, each detected SMBGC was manually curated. This involved rechecking the cluster boundaries, gene content, domain architecture, and alignment against the MIBiG database and previously characterized clusters to ensure functional accuracy. Pan and core genome analysis of available *Xenorhabdus* strains, retrieved from the NCBI database (S1 Table), was performed using Roary [25] and visualized using python.

## Results

### Isolation of entomopathogenic bacteria

During the baiting process, *G. mellonella* larvae exhibited mortality; however, no EPNs emerged from the dead larvae. Despite the absence of visible nematode emergence, a symbiotic bacterial strain, designated MEL2.2, was successfully isolated. After 72 hours of incubation, the strain developed distinct blue-green colonies on NBTA agar (Fig 1). Based on colony morphology and coloration, MEL 2.2 is likely a member of the genus *Xenorhabdus*, as *Xenorhabdus* species are known to produce blue colonies on NBTA agar [26].

### Genome sequencing, assembly and annotation

To characterize the genetic features of strain MEL2.2, its genome was sequenced using Illumina sequencing technology and submitted to NCBI (under the accession ID: SUB15210798). Assembly of the reads revealed the genome of strain MEL2.2 consisted of 284 contigs with an estimated total length of 4.4 Mbp, and an average G + C content of 44.8%. Annotation of the assembled genome predicted a total of 79 tRNA and 4 rRNA genes, with no plasmids detected. The genome comprises 3,823 coding sequences (CDS), of which 2,449 were functionally annotated, while 1,374 were classified as hypothetical proteins (Table 1). Taxonomic classification of strain MEL2.2 was performed using 16S sequence revealed 99% identity to *Xenorhabdus bovienii* T228.

Functional classification using eggNOG categories identified key gene categories (Fig 2 and S2 Table). Notably 290 proteins (8.24%) are involved in replication, recombination, and repair, while 260 proteins (7.39%) are associated with transcription. In addition, 213 proteins (6.05%) contribute to cell wall, membrane, and envelope biogenesis.

The genome also harbours 94 genes (2.67% of the total genome) related to virulence and symbiosis, which are primarily involved in toxin secretion, antibiotic resistance, and stress adaptation. Key virulence factors include resistance-nodulation-cell division (RND) efflux pumps (e.g., *mdtC*, *mdtB*), which contribute to multidrug resistance, and type I secretion system (T1SS) genes like *rtxD*, responsible for exporting toxins. Additionally, several toxin-antitoxin (TA) systems

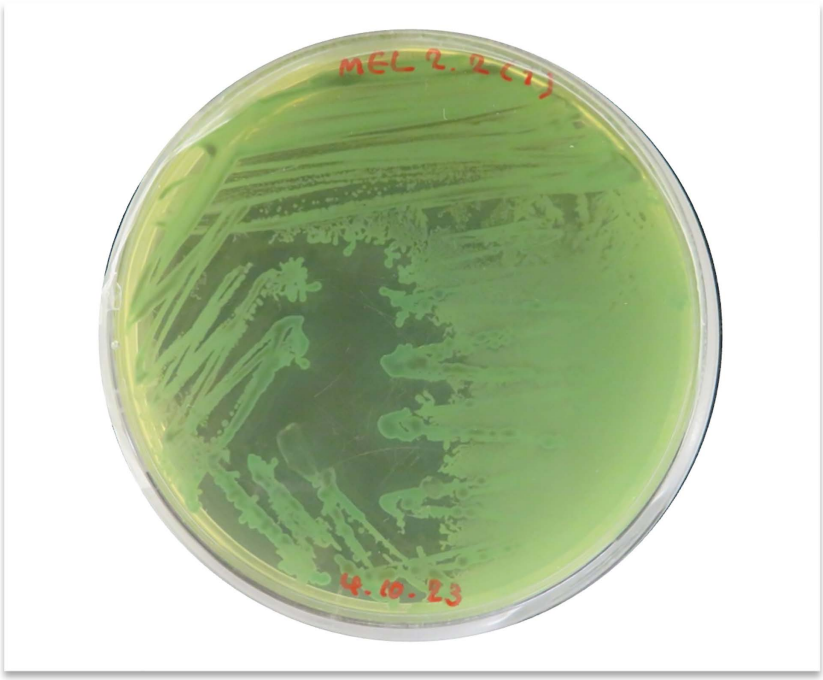



**Fig 1. Colony characteristics of strain MEL 2.2 growing on NBTA.**

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**Table 1. Genetic features of the *de novo* genome of the *Xenorhabdus bovienii* strain MEL 2.2.**

| Genome | *Xenorhabdus bovienii* strain MEL 2.2 |
|---|---|
| Taxonomy | Bacteria; Pseudomonadota; Gammaproteobacteria; Enterobacterales; Morganellaceae; *Xenorhabdus*; *Xenorhabdus bovienii*; *Xenorhabdus bovienii* MEL 2.2 |
| Size | 4.4 Mb |
| GC Content | 44.8% |
| Contigs | 284 |
| Contigs (>= 0 bp) | 332 |
| Contigs (>= 1000 bp) | 248 |
| Largest contig | 1,24,360 |
| N50 | 32784 |
| N90 | 8846 |
| L50 | 39 |
| L90 | 133 |

https://doi.org/10.1371/journal.pone.0331132.t001

such as RelE, ParE, and MazF were identified, which play a role in bacterial persistence under stress conditions. Additionally, genes linked to insecticidal toxin production such as *tcaC*, a component of the toxin complex (Tc) [27], were identified, indicating potential involvement in insect pathogenesis.

### Antimicrobial and secondary metabolite potential

To investigate the biosynthetic gene clusters (BGCs) in *X. bovienii* strain MEL2.2, its genome was analyzed using AntiSMASH version 7.0 [24]. This analysis revealed 15 biosynthetic gene clusters (BGCs) spanning multiple secondary

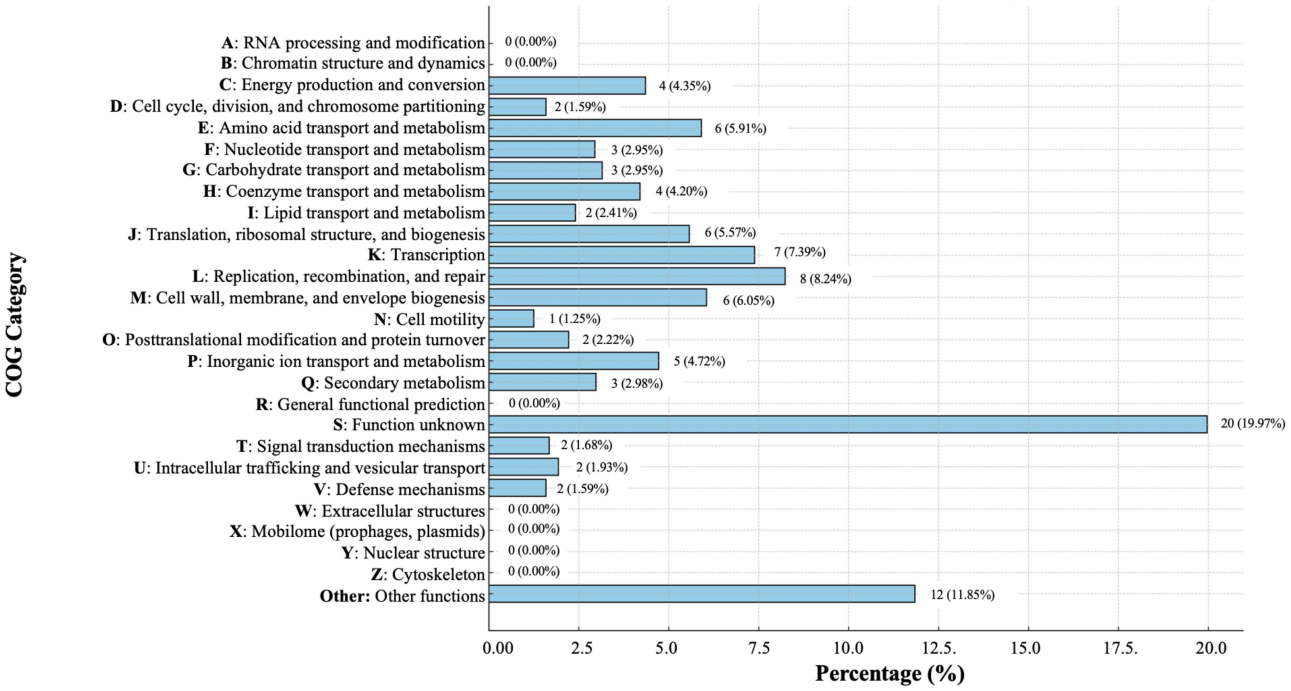



**Fig 2. Distribution of gene features of *Xenorhabdus bovienii* strain MEL 2.2.** The eggNOG functional classification statistical chart of functional genes shows the distribution of genes across different functional groups, represented from A to Z, along with their respective percentages.

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metabolite classes (Table 2). Comparative analysis using MIBiG and manual curation identified both known and novel biosynthetic pathways.

Seven non-ribosomal peptide synthetase (NRPS) clusters were detected in the MEL 2.2 genome, exhibiting varying homology to known clusters (Table 2). These include clusters associated with the production of pyrrolizixenamide A [28], taxlllaid A [29], xefoampeptides A-G [4], trichrysobactin [30], holomycin [31], peptide-antimicrobial-*Xenorhabdus* (PAX) [32], and bovienimide A [33]. Among these, Cluster 9, predicted by antiSMASH as a NRPS, was identified as a putative peptide-antimicrobial-*Xenorhabdus* (PAX) cluster. This assignment was confirmed by manual curation. The predicted domain architecture of the NRPS enzymes within the cluster was re-examined and validated through comparison with previously characterized PAX clusters in *Xenorhabdus* spp. [32,34] which typically contains seven NRPS modules that together encode the biosynthesis of a heptapeptide. Each module includes canonical adenylation (A), thiolation (T), and condensation (C) domains, with some modules containing dual-function condensation/epimerization (C/E) domains. A terminal thioesterase (TE) domain catalyzes product release [32].

Additionally, the genome contains a polyketide cluster (Cluster 12) associated with fabclavine-polyamine (90% similarity) [35,36], and an NI-siderophore cluster (Cluster 13) linked to putrebactin/avaroferrin (90% similarity) [37]. In addition to these characterized clusters, six BGCs remain unclassified. Cluster 2 are small BGCs containing only three domains. Cluster 4 encodes an unknown thiopeptide with the *pflB* gene, which encodes pyruvate-formate lyase (PFL), a key enzyme linked to bacterial metabolism and virulence [38]. Cluster-8 and cluster-11 also lack similarity to any known BGC, suggesting novel biosynthetic potential. Meanwhile, Cluster 14 and Cluster 15 correspond to a betalactone-type cluster and a PpyS-KS cluster, respectively [24]. However, no characterized BGC with substantial homology was identified in MIBiG or KnownClusterBlast, and thus these clusters were classified as unknown.

**Table 2. The secondary metabolites of the *Xenorhabdus bovienii* strain MEL2.2.**

| Cluster | Type | Locus tag of core biosynthetics gene | Most similar known cluster | Identification Approaches | % Similarity | MIBiG accession/ References |
|---|---|---|---|---|---|---|
| Cluster-1 | NRPS | DMG_00274 | Pyrrolizixenamide A | antiSMASH (MIBiG comparison) | 43% | BGC0001873 |
| Cluster-2 | NRPS-like | DMG_00476 | Unknown | | | |
| Cluster-3 | NRPS | DMG_00674, DMG_00675 | Taxlllaid A | antiSMASH (MIBiG comparison) | 100% | BGC0001133 |
| Cluster-4 | Other | DMG_00717, DMG_00720 | Unknown Thiopeptide | | | |
| Cluster-5 | NRPS | DMG_00992 | Xefoampeptides A-G | antiSMASH (Known Cluster Blast) | 33% | BGC0001824 |
| Cluster-6 | NRPS | DMG_01272 | Trichrysobactin | antiSMASH (Known Cluster Blast) | 92% | BGC0002414 |
| Cluster-7 | NRPS | DMG_01535 | Holomycin | antiSMASH (MIBiG comparison) | 80% | BGC0002412 |
| Cluster-8 | NRPS | DMG_01707 - DMG_01709 | Unknown | | | |
| Cluster-9 | NRPS | DMG_01865, DMG_01866, DMG_01867 | PAX | manual curation | | [32,34] |
| Cluster-10 | NRPS | DMG_02050 | Bovienimide A | antiSMASH (MIBiG comparison) | 100% | BGC0002135 |
| Cluster-11 | NRPS, T1PKS | DMG_02333 - DMG_02340 | Unknown | | | |
| Cluster-12 | PKS | DMG_02555, DMG_02556 | Fabclavine-polyamine | antiSMASH (MIBiG comparison) | 90% | BGC0001872 |
| Cluster-13 | Other | DMG_02814, DMG_02822 | Putrebactin/ avaroferrin | antiSMASH (Known Cluster Blast) | 90% | BGC0001870 |
| Cluster-14 | Other (Betalactone) | DMG_03047, DMG_03049 | Unknown | | | |
| Cluster-15 | Other (PpyS-KS) | DMG_03163 | Unknown | | | |

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### Comparative analysis

To assess the genetic diversity among *X. bovienii* strains, a pan-genome analysis was conducted across 61 strains, including MEL2.2, using Roary [25]. The analysis identified four phylogenetic groups (Fig 3), revealing a total of 21,602 genes, highlighting the extensive genetic diversity within the species. Among these, 1,998 genes were classified as core genes, which are highly conserved across most strains, while 19,604 genes were categorized as accessory genes. These accessory genes were further divided into soft core, shell, and cloud genes, reflecting different levels of conservation (Fig 4) (Table 3).

Strain MEL2.2 shares a significant number of core genes with other *X. bovienii* strains but also possesses unique genes not found in several isolates (S3 Table). Notably, MEL2.2 exhibited considerable overlap with the *X. bovienii* strains SS-2004 and feltiae Floridam but lacked certain genes present in strains such as kraussei Becker Underwood and Jollieti. These genetic differences may contribute to niche-specific adaptations and functional variation within the species.

## Discussion

Successful isolation of strain MEL2.2 and subsequent identification revealed the strain to be a member of the species *X. bovienii*. Several *X. bovienii* strains have been isolated from various regions of the world, including Tasmania, Australia, the Czech Republic and Missouri, USA. While some of these isolates, such as SS-2004 from Missouri exhibited virulence against *Manduca sexta* Linnaeus, 1763 [39] and other insect species, others, such as CS03 from the Czech Republic

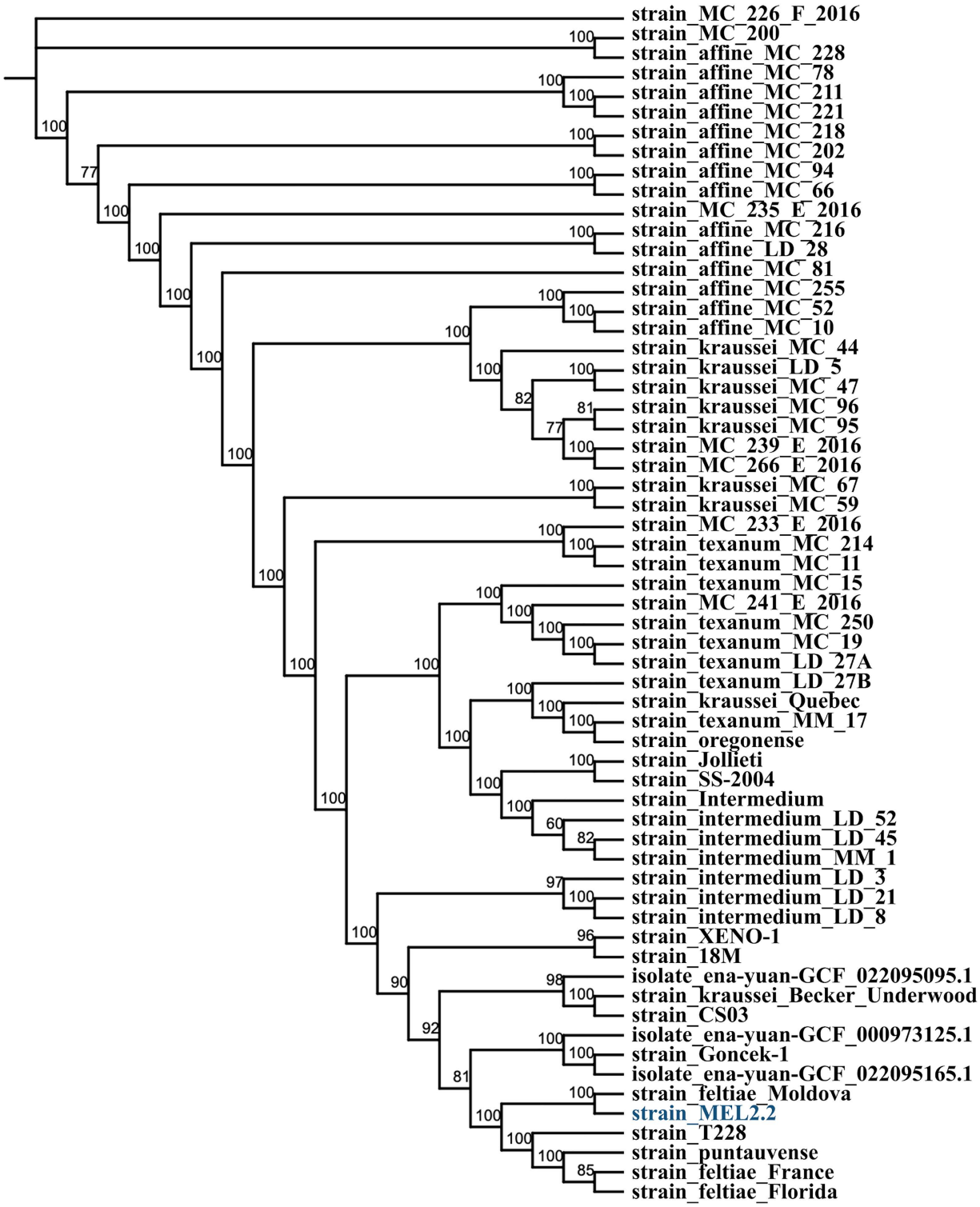



**Fig 3. A phylogenetic tree of *Xenorhabdus bovienii* strains based on core gene alignment results from Roary program with 1,000 bootstraps.**

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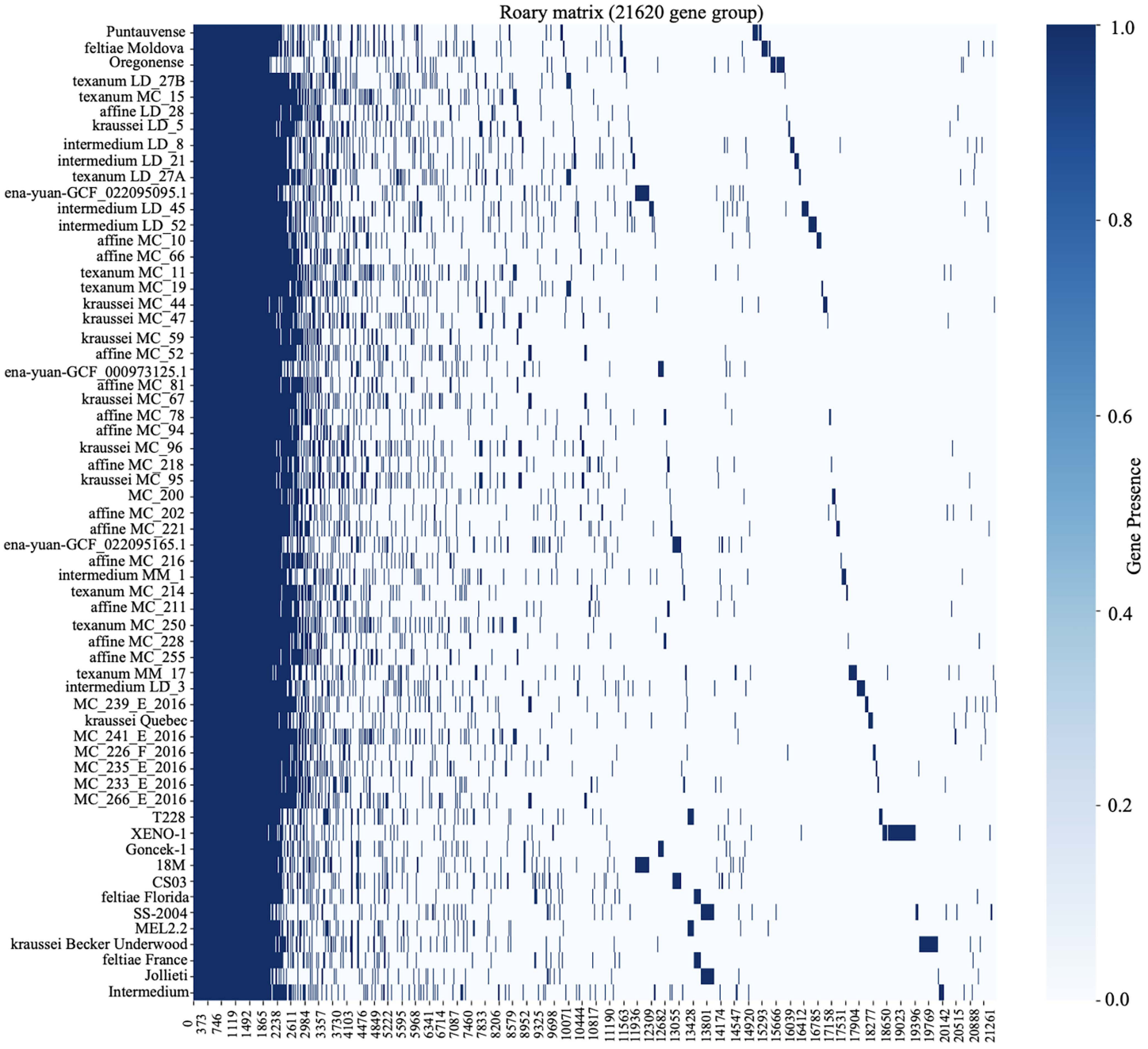



**Fig 4. The pan and core genome heatmap of *Xenorhabdus bovienii* illustrates gene presence and absence across all strains.** The x-axis represents orthologous groups, while the y-axis lists strains. The heatmap categorizes genes into core genes (highly conserved across strains), soft core genes (present in most but not all strains), shell genes (moderately conserved), and cloud genes (strain-specific or singletons). The distinct gene presence patterns suggest evolutionary relationships, genomic plasticity, and potential strain-specific adaptations, which may contribute to niche specialization, host interactions, or metabolic diversity.

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**Table 3. Summary of gene distribution across different categories based on their presence in all *Xenorhabdus bovienii* strains.**

| Gene Category | Description | Number of Genes |
|---|---|---|
| Core genes | (99% <= strains <= 100%) | 1,998 |
| Soft core genes | (95% <= strains < 99%) | 224 |
| Shell genes | (15% <= strains < 95%) | 3,122 |
| Cloud genes | (0% <= strains < 15%) | 16,258 |
| Total genes | (0% <= strains <= 100%) | 21,602 |

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were avirulent in insects [40,41]. Interestingly, *X. bovienii* strain MEL2.2 and these strains shared a high level of genetic similarity, suggesting there may be key genomic features influencing their biological functions, despite their isolation on different continents. Furthermore, their geographical origins also suggest potential adaptations to different environments and the broad dispersal and adaptation of *X. bovienii* worldwide. Further comparative genomic analysis is needed to determine the functional implications of these genetic relationships, particularly regarding insect pathogenicity and secondary metabolite production.

The key genome characteristics of *X. bovienii* strain MEL2.2 (genome size of approximately 4.4 Mbp, G + C content 44.8%), align well with the genomic characteristics of other *Xenorhabdus* species [40,42]. The genome encodes 3,823 coding sequences (CDS), of which 2,449 have functional annotations, while 1,374 (35.9%) are classified as hypothetical proteins. This large complement of hypothetical proteins suggests the presence of uncharacterized genes, which may contribute to unique metabolic pathways and ecological adaptation [43]. The identification of 79 tRNA and 4 rRNA genes highlights the strain's robust translational capacity [44], while the absence of plasmids suggests that key virulence and metabolic traits are encoded within the chromosome [45].

Functional annotation indicated a notable enrichment in genes associated with replication, recombination, and repair processes (8.24%), suggesting a role in maintaining genomic integrity and facilitating adaptation under environmental stress conditions. [46,47]. Genes linked to transcription (7.39%) and to the biogenesis of the cell wall, membrane, and envelope (6.05%) highlight the strain's capacity for dynamic gene regulation and preservation of structural integrity. Furthermore, 2.67% of the genome is associated with virulence and symbiosis, including genes encoding toxin-antitoxin systems, resistance-nodulation-cell division (RND) efflux pumps, and type I secretion systems (T1SS). These characteristics suggested that *X. bovienii* strain MEL2.2 possesses key traits that support persistence in various environments, potential antibiotic resistance, and the ability to interact with or manipulate its host [27,48].

The discovery of 15 biosynthetic gene clusters (BGCs) in *X. bovienii* strain MEL2.2 underscores its broad potential for secondary metabolites production. Among these, several known BGCs correspond to previously characterized compounds such as pyrrolizixenamide A, taxlllaid A, xefoampeptides A-G, PAX, and bovienimide A, which known for their antimicrobial effects, insecticidal properties, and contributions to symbiotic interactions [4,8]. Moreover, the presence of trichrysobactin suggested that MEL 2.2 harbors iron uptake, which are likely essential for its survival and virulence during insect infection. The ability to produce a wide range of antimicrobial compounds also enhance the bacterium's effectiveness as a biocontrol agent by suppressing insect pathogens and microbial competitors. Additionally, the detection of the fabclavine-polyamine cluster, ts potent antimicrobial activity [35], further supports this potential as an effective biocontrol agent. The presence of putrebactin/avaroferrin BGC, previously reported in marine bacteria, implied that the strain possesses mechanisms for iron acquisition, which is vital for survival in iron-restricted environments like insect hemolymph [37,49].

Notably, the identification of six uncharacterized BGCs (Clusters 2, 4, 8, 11, 14, and 15) pointed to the potential for discovering novel secondary metabolites from strain MEL 2.2. Interestingly, cluster 4 encodes an unknown thiopeptide and includes the *pflB* gene, which is associated with pyruvate-formate lyase—an enzyme linked to bacterial metabolism and

virulence [38]. This suggests a possible role in energy metabolism and stress adaptation, which may enhance bacterial survival under host-imposed stress conditions.

While antiSMASH provides valuable predictions of biosynthetic potential, experimental confirmation through metabolomics, gene expression analysis, or compound isolation remains essential to verify whether these BGCs are functionally active and under what conditions they are expressed. Previous studies have demonstrated that many BGCs, especially cryptic ones, require specific environmental cues or genetic activation to produce detectable metabolites [50–52]. Therefore, further research integrating metabolomic profiling, expression studies, and heterologous expression will be necessary to validate and characterize the natural products encoded by the BGCs identified in strain MEL2.2.

The pan and core genome analysis of *X. bovienii* highlights both conserved and unique genomic features. The presence of 1,998 core genes across 61 *X. bovienii* show the essential features that are required for members of this species, while three is significant variability in the accessory genome among different strains. This is most likely due to adaptation in diverse ecological niches among the different strains [53], and in particular, the metabolic pathways and hypothetical proteins present in MEL 2.2 suggest potential roles in specialized functions, possibly shaped by environmental pressures or host interactions. The similarity between *X. bovienii* strain MEL2.2 and *X. bovienii* strains such as SS-2004 and feltiae Florida points to shared ecological niches or evolutionary pressures despite geographic separation, indicating possible evolutionary relationships or functional convergence [53,54]. In contrast, variations observed when compared to strains such as kraussei Becker Underwood and Jollieti emphasize the genetic diversity present among different strains. This underscores the significant role of accessory genes in promoting strain-specific adaptation and diversity. Additional experimental studies focusing on the unique genes of MEL2.2 could provide valuable insights into its ecological functions and evolutionary background.

## Conclusion

In conclusion, genomic analysis of *X. bovienii* strain MEL2.2 revealed its genetic capacity for metabolism, virulence, and secondary metabolite production. Genes related to toxins, secretion systems, and antimicrobial biosynthesis support its dual as an insect pathogen and a nematode symbiont. Analysis of the pan and core genomes further emphasizes a conserved core genome essential for fundamental biological functions, alongside an accessory genome that provides strain-specific variability that contributes to adaptation to diverse ecological niches. These findings deepen our understanding of the strain's ecological significance and underscore its promise for applications in biocontrol and biotechnology. Further investigation into the numerous hypothetical proteins and unexplored biosynthetic gene clusters could reveal novel characteristics and bioactive compounds with valuable practical uses.

### Declaration of Generative AI in Scientific Writing

The author used an AI-assisted tool (GPT-4 Omni) in some parts of the manuscript to correct English grammar.

## Supporting information

**S1 Table. *Xenorhabdus bovienii* genomes used in this study.**
(XLSX)

**S2 Table. Gene function of *Xenorhabdus bovienii* strain MEL 2.2 using the EggNOG 5.0-mapper tool.**
(XLSX)

**S3 Table. Pan and core genome analysis of 61 *Xenorhabdus bovienii* strains, including strain MEL2.2.**
(XLSX)

## Author contributions

**Conceptualization:** Sacha J. Pidot, Aunchalee Thanwisai.

**Data curation:** Wipanee Meesil, Sacha J. Pidot, Aunchalee Thanwisai.

**Formal analysis:** Wipanee Meesil, Liam K. R. Sharkey, Sacha J. Pidot, Aunchalee Thanwisai.

**Funding acquisition:** Wipanee Meesil, Aunchalee Thanwisai.

**Investigation:** Wipanee Meesil, Aunchalee Thanwisai.

**Methodology:** Wipanee Meesil, Aunchalee Thanwisai.

**Project administration:** Aunchalee Thanwisai.

**Resources:** Sacha J. Pidot, Aunchalee Thanwisai.

**Software:** Wipanee Meesil, Liam K. R. Sharkey, Aunchalee Thanwisai.

**Supervision:** Sacha J. Pidot, Aunchalee Thanwisai.

**Validation:** Aunchalee Thanwisai.

**Visualization:** Sacha J. Pidot, Aunchalee Thanwisai.

**Writing – original draft:** Wipanee Meesil, Liam K. R. Sharkey, Sacha J. Pidot, Aunchalee Thanwisai.

**Writing – review & editing:** Wipanee Meesil, Sacha J. Pidot, Aunchalee Thanwisai.

## References


1. Hatting JL, Moore SD, Malan AP. Microbial control of phytophagous invertebrate pests in South Africa: Current status and future prospects. J Invertebr Pathol. 2019;165:54–66. https://doi.org/10.1016/j.jip.2018.02.004 PMID: 29427636
2. Malan AP, Nguyen KB, Addison MF. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from the southwestern parts of South Africa. Afr Plant Prot. 2006;12(1):65–9.
3. Waterfield NR, Ciche T, Clarke D. Photorhabdus and a host of hosts. Annu Rev Microbiol. 2009;63:557–74. https://doi.org/10.1146/annurev.micro.091208.073507 PMID: 19575559
4. Tobias NJ, Wolff H, Djahanschiri B, Grundmann F, Kronenwerth M, Shi Y-M, et al. Natural product diversity associated with the nematode symbionts Photorhabdus and Xenorhabdus. Nat Microbiol. 2017;2(12):1676–85. https://doi.org/10.1038/s41564-017-0039-9 PMID: 28993611
5. Kang SJ, Zhao L, Wang H, Gao JM, Qi J. Chemical structures, biological activities, and biosynthetic analysis of secondary metabolites of the Diatrypaceae family: A comprehensive review. Mycology. 2024;15(3):322–44. https://doi.org/10.1080/21501203.2024.2341648 PMID: 39247891
6. Lin C, Feng XL, Liu Y, Li ZC, Li XZ, Qi J. Bioinformatic analysis of secondary metabolite biosynthetic potential in pathogenic *Fusarium*. J Fungi (Basel). 2023;9(8). https://doi.org/10.3390/jof9080850 PMID: 37623621
7. Nguyen K, Hunt D. Entomopathogenic nematodes: Systematics, phylogeny and bacterial symbionts. Brill. 2007.
8. Boemare NE, Akhurst RJ, Mourant RG. DNA Relatedness between Xenorhabdus spp. (Enterobacteriaceae), Symbiotic Bacteria of Entomopathogenic Nematodes, and a Proposal To Transfer Xenorhabdus luminescens to a New Genus, Photorhabdus gen. nov. International Journal of Systematic Bacteriology. 1993;43(2):249–55. https://doi.org/10.1099/00207713-43-2-249
9. Peel MM, Alfredson DA, Gerrard JG, Davis JM, Robson JM, McDougall RJ, et al. Isolation, identification, and molecular characterization of strains of Photorhabdus luminescens from infected humans in Australia. J Clin Microbiol. 1999;37(11):3647–53. https://doi.org/10.1128/JCM.37.11.3647-3653.1999 PMID: 10523568
10. Gerrard JG, McNevin S, Alfredson D, Forgan-Smith R, Fraser N. *Photorhabdus* species: bioluminescent bacteria as emerging human pathogens? Emerg Infect Dis. 2003;9(2):251–4. https://doi.org/10.3201/eid0902.020222 PMID: 12603999
11. Poinar GO. Taxonomy and biology of Steinernematidae and Heterorhabditidae. 1st ed ed. CRC Press. 1990.
12. Machado RAR, Malan AP, Abolafia J, Ewany J, Bhat AH, Stock SP. *Photorhabdus viridis* sp. nov. Isolated from Heterorhabditis zealandica Entomopathogenic Nematodes. Curr Microbiol. 2024;81(12):423. https://doi.org/10.1007/s00284-024-03935-y PMID: 39443328
13. Bedding RA, Akhurst RJ. A simple technique for the detection o f insect paristic rhabditid nematodes in soil. Nematol. 1975;21(1):109–10. https://doi.org/10.1163/187529275x00419
14. Linnaeus Cv. Systema Naturae per regna tria naturae. Secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio. 1758;1(10):823.
15. Grewal PS, Ehlers R-U, Shapiro-Ilan DI. Nematodes as biocontrol agents: Cabi publishing; 2005.

16. Shapiro-Ilan DI, Gaugler R. Production technology for entomopathogenic nematodes and their bacterial symbionts. J Ind Microbiol Biotechnol. 2002;28(3):137–46. https://doi.org/10.1038/sj.jim.7000230 PMID: 12074087
17. de Sena Brandine G, Smith AD. Falco: high-speed FastQC emulation for quality control of sequencing data. F1000Res. 2019;8:1874. https://doi.org/10.12688/f1000research.21142.2 PMID: 33552473
18. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20. https://doi.org/10.1093/bioinformatics/btu170 PMID: 24695404
19. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19(5):455–77. https://doi.org/10.1089/cmb.2012.0021 PMID: 22506599
20. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30(14):2068–9. https://doi.org/10.1093/bioinformatics/btu153 PMID: 24642063
21. Lu J, Salzberg SL. Ultrafast and accurate 16S rRNA microbial community analysis using Kraken 2. Microbiome. 2020;8(1):124. https://doi.org/10.1186/s40168-020-00900-2 PMID: 32859275
22. Mikheenko A, Prjibelski A, Saveliev V, Antipov D, Gurevich A. Versatile genome assembly evaluation with QUAST-LG. Bioinformatics. 2018;34(13):i142–50. https://doi.org/10.1093/bioinformatics/bty266 PMID: 29949969
23. Huerta-Cepas J, Szklarczyk D, Heller D, Hernández-Plaza A, Forslund SK, Cook H, et al. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res. 2019;47(D1):D309–14. https://doi.org/10.1093/nar/gky1085 PMID: 30418610
24. Blin K, Shaw S, Augustijn HE, Reitz ZL, Biermann F, Alanjary M, et al. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. Nucleic Acids Res. 2023;51(W1):W46–W50. https://doi.org/10.1093/nar/gkad344 PMID: 37140036
25. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, et al. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics. 2015;31(22):3691–3. https://doi.org/10.1093/bioinformatics/btv421 PMID: 26198102
26. Thanwisai A, Tandhavanant S, Saiprom N, Waterfield NR, Ke Long P, Bode HB, et al. Diversity of Xenorhabdus and Photorhabdus spp. and their symbiotic entomopathogenic nematodes from Thailand. PLoS One. 2012;7(9):e43835. https://doi.org/10.1371/journal.pone.0043835 PMID: 22984446
27. Blackburn MB, Domek JM, Gelman DB, Hu JS. The broadly insecticidal Photorhabdus luminescens toxin complex a (Tca): activity against the Colorado potato beetle, Leptinotarsa decemlineata, and sweet potato whitefly, Bemisia tabaci. J Insect Sci. 2005;5:32. https://doi.org/10.1093/jis/5.1.32 PMID: 17119614
28. Schimming O, Challinor VL, Tobias NJ, Adihou H, Grün P, Pöschel L, et al. Structure, Biosynthesis, and Occurrence of Bacterial Pyrrolizidine Alkaloids. Angew Chem Int Ed Engl. 2015;54(43):12702–5. https://doi.org/10.1002/anie.201504877 PMID: 26465655
29. Kronenwerth M, Bozhüyük KAJ, Kahnt AS, Steinhilber D, Gaudriault S, Kaiser M, et al. Characterisation of taxllaids A-G; natural products from Xenorhabdus indica. Chemistry. 2014;20(52):17478–87. https://doi.org/10.1002/chem.201403979 PMID: 25351611
30. Stow PR, Reitz ZL, Johnstone TC, Butler A. Genomics-driven discovery of chiral triscatechol siderophores with enantiomeric Fe(iii) coordination. Chem Sci. 2021;12(37):12485–93. https://doi.org/10.1039/d1sc03541j PMID: 34603680
31. Zhang S-D, Isbrandt T, Lindqvist LL, Larsen TO, Gram L. Holomycin, an Antibiotic Secondary Metabolite, Is Required for Biofilm Formation by the Native Producer Photobacterium galatheae S2753. Appl Environ Microbiol. 2021;87(11):e00169–21. https://doi.org/10.1128/AEM.00169-21 PMID: 33771780
32. Watzel J, Duchardt-Ferner E, Sarawi S, Bode HB, Wöhnert J. Cooperation between a T Domain and a Minimal C-Terminal Docking Domain to Enable Specific Assembly in a Multiprotein NRPS. Angew Chem Int Ed Engl. 2021;60(25):14171–8. https://doi.org/10.1002/anie.202103498 PMID: 33876501
33. Li J-H, Cho W, Hamchand R, Oh J, Crawford JM. A Conserved Nonribosomal Peptide Synthetase in Xenorhabdus bovienii Produces Citrulline-Functionalized Lipopeptides. J Nat Prod. 2021;84(10):2692–9. https://doi.org/10.1021/acs.jnatprod.1c00573 PMID: 34581573
34. Dreyer J, Rautenbach M, Booysen E, van Staden AD, Deane SM, Dicks LMT. Xenorhabdus khoisanae SB10 produces Lys-rich PAX lipopeptides and a Xenocoumacin in its antimicrobial complex. BMC Microbiol. 2019;19(1):132. https://doi.org/10.1186/s12866-019-1503-x PMID: 31195965
35. Fuchs SW, Grundmann F, Kurz M, Kaiser M, Bode HB. Fabclavines: bioactive peptide-polyketide-polyamino hybrids from Xenorhabdus. Chembiochem. 2014;15(4):512–6. https://doi.org/10.1002/cbic.201300802 PMID: 24532262
36. Hancock RE. Cationic peptides: effectors in innate immunity and novel antimicrobials. Lancet Infect Dis. 2001;1(3):156–64. https://doi.org/10.1016/S1473-3099(01)00092-5 PMID: 11871492
37. Hirschmann M, Grundmann F, Bode HB. Identification and occurrence of the hydroxamate siderophores aerobactin, putrebactin, avaroferrin and ochrobactin C as virulence factors from entomopathogenic bacteria. Environ Microbiol. 2017;19(10):4080–90. https://doi.org/10.1111/1462-2920.13845 PMID: 28654175
38. Mucci NC. Mechanisms by which Xenorhabdus nematophila interacts with hosts using integrated -omics approaches. University of Tennessee. 2022. https://trace.tennessee.edu/utk_graddiss/7100
39. GBIF Secretariat. GBIF Backbone Taxonomy. https://doi.org/10.15468/39omei. 2023.

40. Chaston JM, Suen G, Tucker SL, Andersen AW, Bhasin A, Bode E, et al. The entomopathogenic bacterial endosymbionts Xenorhabdus and Photorhabdus: convergent lifestyles from divergent genomes. PLoS One. 2011;6(11):e27909. https://doi.org/10.1371/journal.pone.0027909 PMID: 22125637

41. Sugar DR, Murfin KE, Chaston JM, Andersen AW, Richards GR, deLéon L, et al. Phenotypic variation and host interactions of Xenorhabdus bovienii SS-2004, the entomopathogenic symbiont of Steinernema jollieti nematodes. Environ Microbiol. 2012;14(4):924–39. https://doi.org/10.1111/j.1462-2920.2011.02663.x PMID: 22151385

42. Murfin KE, Whooley AC, Klassen JL, Goodrich-Blair H. Comparison of Xenorhabdus bovienii bacterial strain genomes reveals diversity in symbiotic functions. BMC Genomics. 2015;16:889. https://doi.org/10.1186/s12864-015-2000-8 PMID: 26525894

43. Shahbazi M, Tohidfar M, Azimzadeh Irani M, Moheb Seraj RG. Functional annotation and evaluation of hypothetical proteins in cyanobacterium Synechocystis sp. PCC 6803. Biocatalysis and Agricultural Biotechnology. 2022;39:102246. https://doi.org/10.1016/j.bcab.2021.102246

44. Raina M, Ibba M. tRNAs as regulators of biological processes. Front Genet. 2014;5:171. https://doi.org/10.3389/fgene.2014.00171 PMID: 24966867

45. Tailliez P, Laroui C, Ginibre N, Paule A, Pagès S, Boemare N. Phylogeny of Photorhabdus and Xenorhabdus based on universally conserved protein-coding sequences and implications for the taxonomy of these two genera. Proposal of new taxa: X. vietnamensis sp. nov., P. luminescens subsp. caribbeanensis subsp. nov., P. luminescens subsp. hainanensis subsp. nov., P. temperata subsp. khanii subsp. nov., P. temperata subsp. tasmaniensis subsp. nov., and the reclassification of P. luminescens subsp. thracensis as P. temperata subsp. thracensis comb. nov. Int J Syst Evol Microbiol. 2010;60(Pt 8):1921–37. https://doi.org/10.1099/ijs.0.014308-0 PMID: 19783607

46. Cantalapiedra CP, Hernández-Plaza A, Letunic I, Bork P, Huerta-Cepas J. eggNOG-mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. Mol Biol Evol. 2021;38(12):5825–9. https://doi.org/10.1093/molbev/msab293 PMID: 34597405

47. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, von Mering C, et al. Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper. Mol Biol Evol. 2017;34(8):2115–22. https://doi.org/10.1093/molbev/msx148 PMID: 28460117

48. Pletzer D, Weingart H. Characterization and regulation of the resistance-nodulation-cell division-type multidrug efflux pumps MdtABC and MdtUVW from the fire blight pathogen Erwinia amylovora. BMC Microbiol. 2014;14:185. https://doi.org/10.1186/1471-2180-14-185 PMID: 25012600

49. Wang S, Liang H, Liu L, Jiang X, Wu S, Gao H. Promiscuous Enzymes Cause Biosynthesis of Diverse Siderophores in Shewanella oneidensis. Appl Environ Microbiol. 2020;86(7):e00030-20. https://doi.org/10.1128/AEM.00030-20 PMID: 32005730

50. Bode E, Assmann D, Happel P, Meyer E, Münch K, Rössel N, et al. easyPACId, a Simple Method for Induced Production, Isolation, Identification, and Testing of Natural Products from Proteobacteria. Bio Protoc. 2023;13(13):e4709. https://doi.org/10.21769/BioProtoc.4709 PMID: 37449040

51. Pillay LC, Nekati L, Makhwitine PJ, Ndlovu SI. Epigenetic Activation of Silent Biosynthetic Gene Clusters in Endophytic Fungi Using Small Molecular Modifiers. Front Microbiol. 2022;13:815008. https://doi.org/10.3389/fmicb.2022.815008 PMID: 35237247

52. Baral B, Akhgari A, Metsä-Ketelä M. Activation of microbial secondary metabolic pathways: Avenues and challenges. Synth Syst Biotechnol. 2018;3(3):163–78. https://doi.org/10.1016/j.synbio.2018.09.001 PMID: 30345402

53. Jaiswal AK, Tiwari S, Tavares GC, da Silva WM, de Castro Oliveira L, Ibraim IC. Pan-omics focused to Crick's central dogma. Pan-genomics: Applications, Challenges, and Future Prospects. Elsevier. 2020:1–41.

54. Giovannoni SJ, Cameron Thrash J, Temperton B. Implications of streamlining theory for microbial ecology. ISME J. 2014;8(8):1553–65. https://doi.org/10.1038/ismej.2014.60 PMID: 24739623