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RESEARCH

Whole genome sequencing and functional analysis of a novel biofilm-eradicating strain *Nocardiopsis lucentensis* EMB25

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Abstract
The process of biofilm formation is intricate and multifaceted, requiring the individual cells to secrete extracellular polymeric substances (EPS) that subsequently aggregate and adhere to various surfaces. The issue of biofilms is a significant concern for public health due to the increased resistance of microorganisms associated with biofilms to antimicrobial agents. The current study describes the whole genome and corresponding functions of a biofilm inhibiting and eradicating actinobacteria isolate identified as *Nocardiopsis lucentensis* EMB25. The *N. lucentensis* EMB25 has 6.5 Mbp genome with 71.62% GC content. The genome analysis by BLAST Ring Image Generator (BRIG) revealed it to be closely related to *Nocardiopsis dassonvillei* NOCA502F. Interestingly, based on orthologous functional groups reflected by average nucleotide identity (ANI) analysis, it was 81.48% similar to *N. arvandica* DSM4527. Also, it produces lanthipeptides and linear azole(in)e-containing peptides (LAPs) akin to *N. arvandica*. The secondary metabolite search revealed the presence of major gene clusters involved in terpene, ectoine, siderophores, Lanthipeptides, RiPP-like, and T1PKS biosynthesis. After 24 h of treatment, the cell-free extract effectively eradicates the pre-existing biofilm of *P. aeruginosa* PseA. Also, the isolated bacteria exhibited antibacterial activity against MRSA, *Staphylococcus aureus* and *Bacillus subtilis* bacteria. Overall, this finding offers valuable insights into the identification of BGCs, which contain enzymes that play a role in the biosynthesis of natural products. Specifically, it sheds light on the functional aspects of these BGCs in relation to *N. lucentensis*.

Keywords Whole-genome · Actinobacteria · Secondary metabolites · Biofilm · Antimicrobial · *Nocardiopsis lucentensis*

Introduction

The global spread of antimicrobial resistance (AMR) is widely recognized as a significant challenge in healthcare and disease management. The World Health Organization has warned that the rise of multidrug-resistant bacteria is putting the effectiveness of antibiotics in jeopardy. Antibiotics have played a crucial role in advancing medical science, but their usefulness is now being threatened. The involvement of biofilms in antimicrobial resistance (AMR) is a crucial factor that substantially contributes to the development of resistance (Costerton et al. 2003). The thick layer of the extracellular polymeric substance (EPS) could potentially hinder the effectiveness of antibiotics that try to penetrate biofilms through diffusion-reaction inhibition. This could occur through the antibiotics binding with EPS to form complexes, or through enzymatic degradation (Billings et al. 2015; Lahiri et al. 2019; Sharma et al. 2019). Therefore, finding biofilm inhibitors that can inhibit the bacterial biofilm mode of AMR could be an important scientific venture. Although chemical synthesis has significantly contributed to antimicrobial compounds, nature remains the richest and primary gateway for discovering new antimicrobial compounds (Samrot et al. 2021).

Actinobacteria are a valuable source of bioactive compounds with antibiotic properties that are highly significant in the pharmaceutical industry (De Simeis & Serra. 2021). Actinobacteria of terrestrial origin have been essential in the quest for novel antibiotics for more than half a centuary. However, the possibility of re-discovering the same compounds presents a significant limitation (Chen et al. 2021). Lately, there has been a lot of interest in screening marine actinobacteria due to their abundance and their potential for

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producing unique bioactive compounds with diverse structures and properties (Eliwa et al. 2017).

Nocardiopsis is a genus that holds significant importance in natural product research within the pharmaceutical and biotechnological industries (Hamed et al. 2018). It is an aerobic, Gram-positive, and halo-tolerant actinobacteria. These organisms can be discovered in a variety of habitats such as terrestrial areas as epiphytes or endophytes, desert soils, and even marine environments. They are typically found in environments that range from moderate to hypersaline (Bennur et al. 2016). In order to survive in harsh environments, they have the ability to produce various extremozymes, surfactants, compatible solutes, and bioactive compounds (Chen et al. 2021).

In recent years, it has become clear that the genus *Nocardiopsis* has a lot of potential for producing therapeutic leads. This is due to their many different chemotypes (Hadj Rabia-Boukhalfa et al. 2017). There are currently almost 22 sequenced genomes from the *Nocardiopsis* genus that have been submitted to NCBI from different marine and terrestrial environments (Eliwa et al. 2017). To predict and discover new secondary metabolites with different frameworks efficiently, it's best to use genomic screening and chemical investigation through chromatographic methods on microbial strains like *Nocardiopsis* (Ibrahim et al. 2018).

Nocardiopsis is responsible for producing around 3% of marine actinomycetes compounds, making it the third largest contributor among actinomycetes. Studies on this genus have found a variety of secondary metabolites with potential bioactivity. These include dopsisamine (Takahashi et al. 1986), lucentamycins A–D (Cho et al. 2007), griseusin D (Li et al. 2007), nocapyrones A–D (Schneemann et al. 2010), nocazoline A (Fu et al. 2011), nocapyrones H–J (Kim et al. 2013), and nocardiopsins A and B (Wu et al. 2013). Recent studies have also discovered other compounds that may have bioactive properties (Shi et al. 2022).

Our previous study concentrated on *N. lucentensis* EMB25's ability to prevent biofilm formation in *P. aeruginosa* strains by analyzing the organic extract and docking identified compounds with RhlR and LasR (Goel et al. 2023). In this study, we have performed the genomic investigation of the *N. lucentensis* EMB25 strain, the first biofilm-inhibiting and eradicating strain. This data has the potential to generate new knowledge in various areas related to medicinal chemistry. These areas may include, the identification and validation of new drug targets in human pathogens, and the discovery of new chemical entities.

Materials and methods

The strain

The bacteria was isolated from salt pan, Pen, Maharashtra, India (18.7358°N, 73.0947° E) and identified as *N. lucentensis* EMB25 by 16S rRNA sequencing. The gene sequence was submitted to NCBI GenBank with the accession number MW582546.1. Currently, our laboratory maintains the strain as EMB25 for continued research and analysis.

The other test pathogenic strains used in the study are following: Gram-positive (*Staphylococcus aureus* ATCC 23235, MRSA ATCC 43300, *Bacillus subtilis*, a lab isolate) and Gram-negative bacteria (*P. aeruginosa* PseA MTCC 10634).

Extraction of genomic DNA of *N. lucentensis* EMB25

A single colony of *N. lucentensis* EMB25 was inoculated in International Streptomyces Project-2 Medium (ISP-2) broth and incubated at 30 °C. The cells were harvested after 7 days of incubation by centrifuging at 8000 x *g* for 10 min. The pellets were suspended, and genomic DNA was isolated using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, USA). The extraction process was carried out in accordance with the manufacturer's instructions. The quality and quantity were accessed using agar gel electrophoresis and Nanodrop 2000 (Thermo Scientific, USA).

Carbon source utilization by *N. lucentensis* EMB25

Carbon source utilization was investigated using KB009 TM HiCarbo Kit (Himedia, India). Inoculation and interpretation of the results were made as per the guidelines of the kit.

Scanning electron microscopy

The morphological characteristics of *N. lucentensis* EMB25 were recorded through scanning electron microscopy (SEM) following the protocol described by Ali et al. (2021). Images were acquired and processed using FESEM (FEI Company, Netherland, Model: FEI Quanta 200 F SEM).

Whole genome sequencing

The whole genome sequencing was performed using Illumina sequencing and the GridION X5, the long-read platforms at Genotypic Technology, Bangalore, India.

The Illumina library preparation was carried out using the QIASeq FX DNA kit as per the instructions mentioned by the manufacturer. Briefly, enzymatically fragmentation

of genomic DNA, end-repairment and a-tailing, all three reactions were simultaneously carried out utilizing the FX Enzyme Mix followed by adapter ligation to generate sequencing libraries. The obtained libraries were then subjected to Indexing-PCR and final extension to enrich the adapter-tagged fragments. After the purification of generated libraries, sequencing was carried out on the Illumina HiSeq X Ten sequencer for 150 cycles.

The genomic DNA was purified, end-paired, and cleaned up, and native barcode ligation was done using NEB blunt/TA ligase to prepare the library for nanopore sequencing. The DNA was quantified, and adaptors were ligated for 15 min using NEBNext Quick Ligation Module and cleaned up using 0.6X AmPure beads. Finally, the library prepared to be sequenced was eluted in 15 µL of elution buffer. Using a SpotON flow cell R9.4 (FLO-MIN106), a 48-hour sequencing technique was used on the GridION X5. Guppy v2.3.4 was used to base-call and de-multiplex nanopore raw reads ('fast5' format).

Data analysis and genome comparison

The generated raw reads were assembled using a unicycler hybrid assembler generated using SPAdes 3.6.2 (Wick et al. 2017) from both Illumina and nanopore platforms. The assembled genome sequence was annotated using the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) v5.2 (Tatusova et al. 2016). The predicted genes were subjected to pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa 2000). The genomic comparison was carried out using BLAST Ring Image Generator (BRIG) v0.95, which uses CGview to generate the circular image and BLAST for genome similarity comparison (Alikhan et al. 2011) and Orthologous Average Nucleotide Identity Tool (OAT) v0.93.1 analysis tool, which uses only orthologous fragments to calculate ANI values to generate heatmap (Lee et al. 2016).

RAST annotation and secondary metabolites prediction

RAST (Rapid Annotation using Subsystem Technology), which was released in 2007, is a fully annotated service for full or almost complete bacterial and archaeal genomes. The genome sequence is submitted either in FASTA or Genbank format to the RAST server. The server takes around 12–24 h to annotate the genome. In the current study, version 2.0 was used to annotate the genome, and for further comparison with other genomes, the SEED platform was used, also to know the subsystem category distribution (Overbeek et al. 2014). Furthermore, anti-SMASH (version 7.0), an online database, was used to predict the secondary metabolites in *N. lucentensis* EMB25. The genome was run to the web based online tool to identify BGCs encoding secondary metabolites (Blin et al. 2023). Based on the findings, inferences were drawn regarding the gene sequence and percent identity. In addition, BActeriocin GEnome mining tooL (BAGEL4), a web service, was used to identify Ribosomally synthesised and Post Translationally Modified Peptides (RiPPs) and antimicrobial peptides (bacteriocins) (van Heel et al. 2018).

Antibacterial activity

The antibacterial activity was performed against Gram-positive (*Staphylococcus aureus*, MRSA, *Bacillus subtilis*) and Gram-negative bacteria (*Pseudomonas aeruginosa* PseA) using agar-plug assay (Ortlieb et al. 2021). The actinobacteria EMB25 was streaked tightly on ISP2 agar media and incubated at 30 °C for seven days. Afterwards, test microorganisms were spread onto nutrient agar media, and a well is then cut to add EMB25 bacteria after sporulation. The plates were incubated at 30 °C for 24 h, and the zone of inhibition was measured. In addition, disc diffusion assay was performed against *S. aureus* and MRSA with the organic extract prepared as mentioned by Goel et al. (2023).

Biofilm eradication activity of *N. lucentensis* EMB25 cell-free extract and confocal microscopy

The biofilm-forming bacteria, *P. aeruginosa* PseA was used to explore the biofilm eradication potential of *N. lucentensis* EMB25. After 24 h of biofilm formation, cell-free extract of *N. lucentensis* EMB25 was added in various volumes (50, 100, 150 and 200 µL) and incubated at 30 °C for 24 h. Thereafter, biofilm quantification was done using crystal violet (CV) assay as described by Balasubramanian et al. (2017), and cell counts were performed to check cell viability. Along with the CV assay, confocal microscopy was performed, as mentioned by Goel et al. (2023). The adherent cells control and test (150 µL) were stained with the Filmtracer™ LIVE/DEAD™ Biofilm Viability Kit (Invitrogen, ThermoFisher Scientific, USA) and visualized under the confocal microscope (Laser Scanning Confocal Microscope: Leica TCS SP8, Germany).

Results

Biochemical characterization and morphology study of *N. lucentensis* EMB25

The *N. lucentensis* EMB25 exhibited a wide range of qualitative carbon source utilization. Of 35 biochemical tests, 32

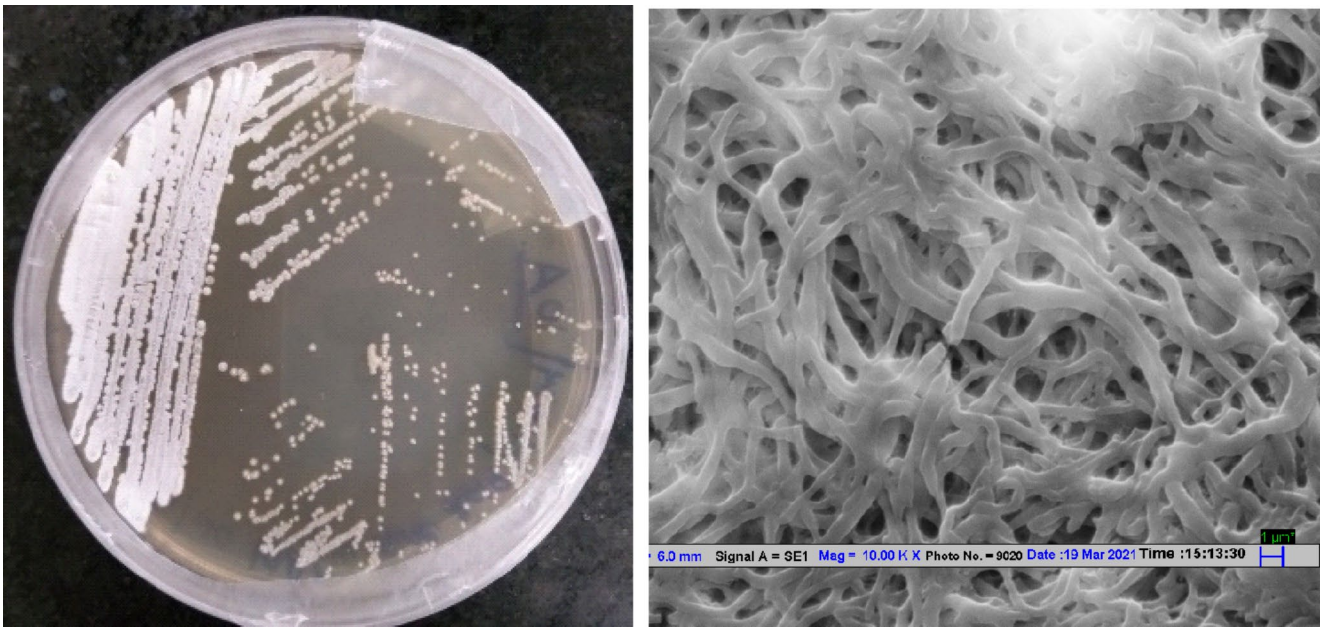


Fig. 1 The colony of *N. lucentensis* EMB25 (**A**) on ISP2 agar plate (**B**) Scanning electron micrographs

Table 1 The assembly statistics of *N. lucentensis* EMB25

Sample Name	*N. lucentensis* EMB25
Contigs Generated	2
Maximum Contig Length	6,511,560
Minimum Contig Length	39,151
Average Contig Length	3275355.5
Median Contig Length	3275355.5
Total Contigs Length	6,550,711
Contigs > = 10 Kbp	2
Contigs > = 1 Mbp	1
N50 value	6,511,560

expressed positive, and 3 expressed negative results. It grew optimally on lactose, xylose, fructose, dextrose, sucrose, and inositol. It was cable of utilizing some of the other carbon sources viz. maltose, galactose, trehalose, melibiose, mannose, adonitol, arabitol, erythritol, alpha-Methyl-D-glucoside, but with relatively less growth. However, it failed to grow on raffinose, al-arabinose, and malonate (Supplementary Table S1).

The SEM images of *N. lucentensis* EMB25 grown on ISP2 agar clearly show the typical actinobacteria-like hyphae network (Fig. 1).

Sequencing, assembly, and genomic comparisons

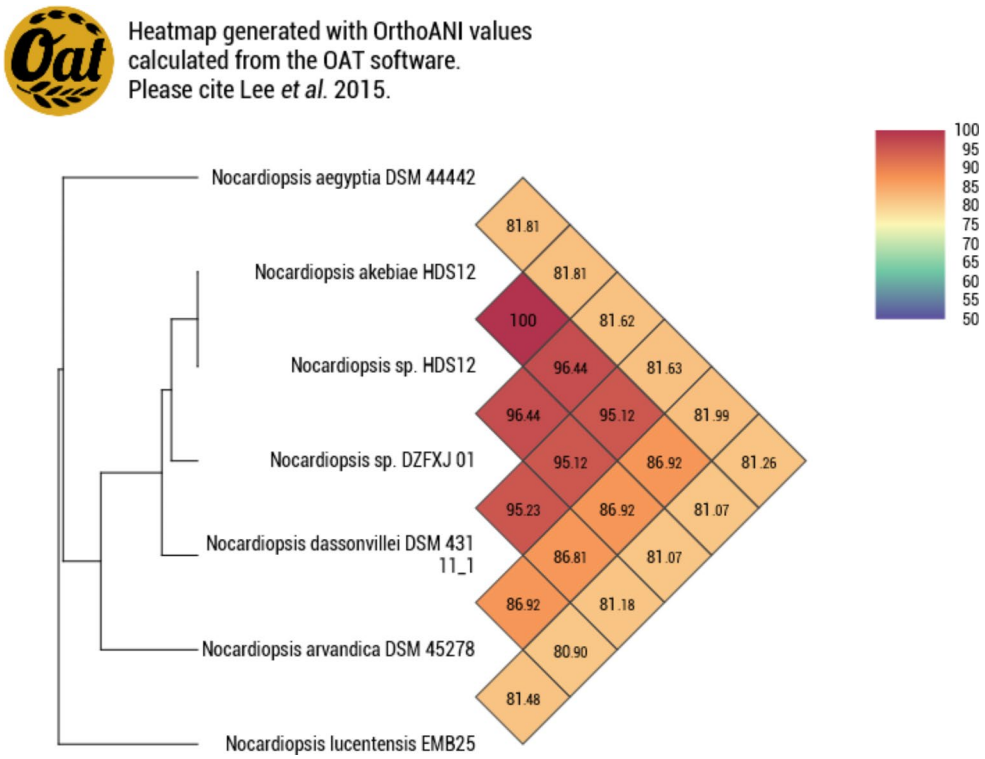
N. lucentensis EMB25 genome is of linear size 6.5 Mbp with 71.6% GC content. Out of 5775 protein-coding sequences, 5393 (93.38%) proteins were annotated using the PROKKA tool (Seemann 2014). The assembly statistics of the obtained sequences are summarized in Table 1. Genomic comparison analysis revealed that *N. lucentensis* EMB25 isolate is closely related to the *Nocardiopsis dassonvillei* NOCA502F (NZ_CP017965.1) (Supplementary Figure S1), which is known to be active against Gram-positive bacteria *Bacillus subtilis*, yeast *Candida albicans* and produces enzymes protease, β-galactosidase, and glucosidase (Yu et al. 2017).

The orthoANI values for each genome comparison with the *N. lucentensis* EMB25 are mentioned in Supplementary Table S2. The maximum value is 81.48 inferring the highest similarity with the *Nocardiopsis arvandica* DSM45278 based on orthologous groups match (Fig. 2).

Genomic prediction and genome annotation

Gene ontology (GO) annotation resulted in the top 5 abundant protein functions: Membrane protein, TetR family transcriptional regulator, Non-specific serine/threonine protein kinase (EC 2.7.11.1), Transcriptional regulator, TetR family, ABC transporter related protein clustered into 10 biological, 10 molecular and 5 cellular branches (Supplementary Figure S2).

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database interpreted that the genes are mainly involved in 27 pathway functions, of which 49 genes are associated with the pathways of metabolism of terpenoids and polyketides responsible for antibiotic synthesis (Fig. 3), namely ansamycins (K00615), enediyne (K20420), Siderosphore (K12240), vancomycin group antibiotics (K16437), along with other potential antibiotics. In addition, 37 genes belong to the pathways of biosynthesis of other secondary metabolites such as novobiocin (K00812), monobactam (K00215),



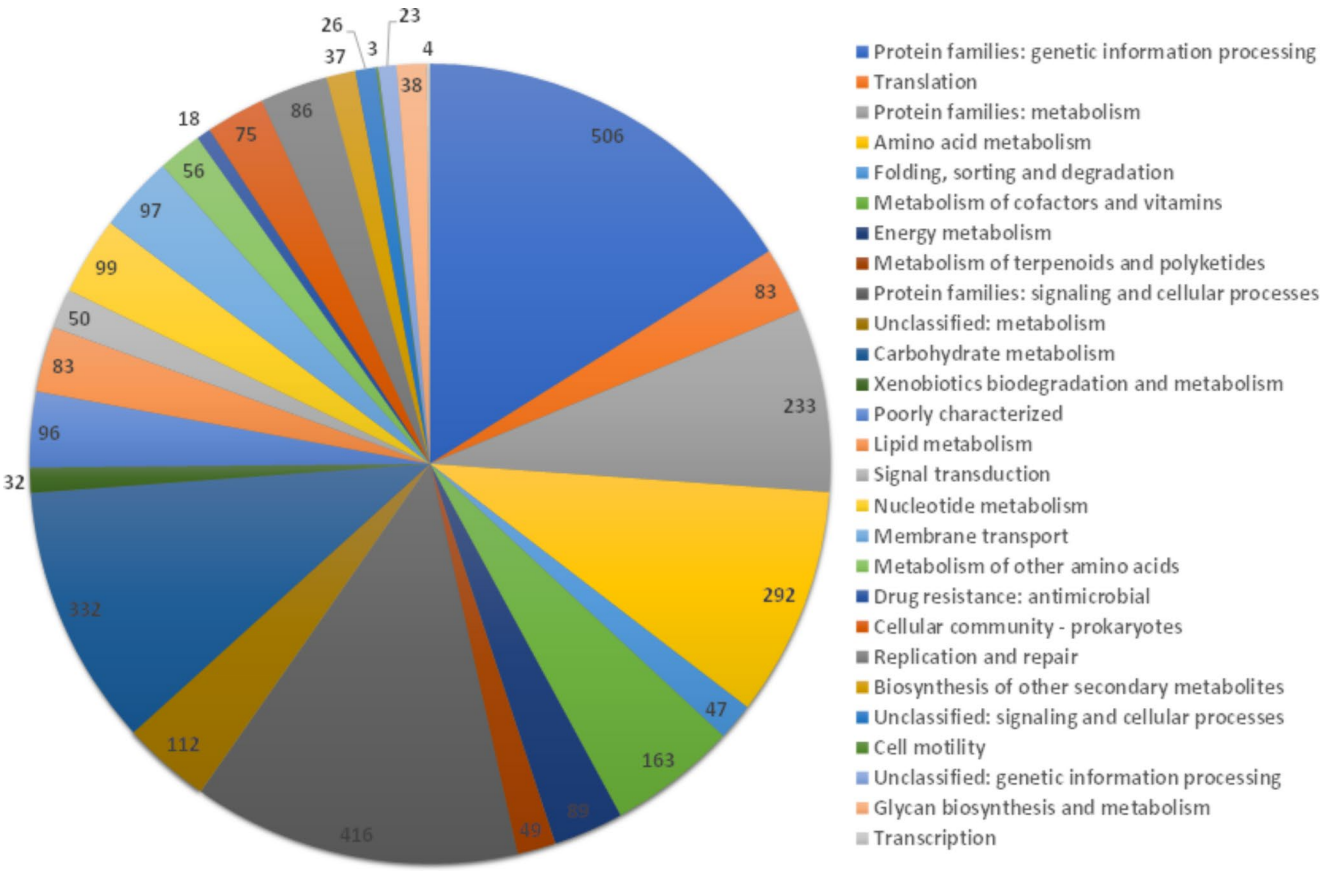


Fig. 2 Heat map depicting genomic comparison of *N. lucentensis* EMB25 based on orthoANI values

Fig. 3 The Kyoto Encyclopaedia of Genes and Genomes (KEGG) function annotation of *N. lucentensis* EMB25

penicillin, cephalosporin (K17836), acarbose, validamycin (K19978), prodigiosin (K00059), streptomycin (K01092), Neomycin, kanamycin and gentamicin (K00845), and carbapenem (K00147) (Supplementary Table S3).

RAST annotation and secondary metabolites prediction

The online antiSMASH database predicted total 24 secondary BGCs regions for NRPS, T1PKS, CDPS, terpene, lanthipeptide class i, iii, iv, RiPP-like, NI-siderophore, LAP, thiopeptide, melanin and ectoine. Region 4 and 6 belongs to terpene and lanthipeptide class-iii, which have shown 100% similarity with isorenieratene and SapB, respectively. Isorenieratene is a carotenoid, and SapB is a peptide used in aerial mycelium formation. Xanthobaccin C was first isolated from *Stenotrophomonas* sp. strain SB-K88 and is an antifungal drug that shows 50% similarity and is associated with region 16. Neocarzinostatin shows 50% similarity and is an antitumor antibiotic produced by *Streptomyces* sp. that mediates strand breaking of DNA. There are many drugs that are produced such as Siamycin, mathermycin, duramycin, neocarzilin A/neocarzilin B, jerangolid A/jerangolid D, streptamidine, nonactin/monactin/dinactin/trinactin/tetranactin, azetidomonamide A/azetidomonamide B, 4-hydroxy-3-nitrosobenzamide, grixazone A, ferroverdin/bagremycin, Rosamicin, carrimycin and ectoine (Table 2).

The SEED viewer environment provided the subsystems classification of the encoded proteins, and only 19% comes under subsystem category distribution. As per RAST annotation, 48 genes are involved in stress response, 19 genes in the production of secondary metabolites and 40 genes in virulence diseases and defence mechanisms (Fig. 4).

BAGEL4 identified lanthipeptides class i and iv, SapB, and lactococcin 972. Lactococcin 972 inhibits cell division by preventing the incorporation of septum cell wall precursor and is the only non-lantibiotic that does not target the cytoplasmic membrane (Martínez et al. 2008).

Bioactivity of cell-free extract of *N. lucentensis* EMB25

The agar-plug assay showed the antibacterial activity against Gram-positive bacteria, namely *S. aureus*, MRSA, and *B. subtilis*. However, no activity was observed against Gram-negative *P. aeruginosa* PseA (Fig. 5A). The organic extract was used for disc diffusion assay and resulted antibacterial activity at 40 and 120 µg/mL against *S. aureus* and MRSA respectively (Supplementary Figure S3).

Furthermore, the effect of *N. lucentensis* EMB25 was investigated for eradication of the preformed biofilm and cell viability (log CFU/mL) (Fig. 5B). The extract eradicated the biofilm in a dose-dependent manner and thereby reduced the viable cell count. In order to validate the above observations, confocal microscopy was performed (Fig. 5C). Two dyes SYTO 9 and Propidium iodide (PI), were used to stain the biofilm. SYTO 9 is used to visualize the live cells and gives green color, while PI stains dead cells and appears red in color. The result (Fig. 5C) clearly shows a significant reduction in biofilm and proportionate killing of bacteria colonizing and inhabiting within the biofilm. The *N. lucentensis* EMB25 secretes potential bioactives that can be used in AMR therapeutics.

Discussion

The *N. lucentensis* EMB25 was isolated from Salt pan, Pen, Maharashtra, India. It is a rare actinobacteria possessing the capability to produce novel bioactive compounds. We have previously published a study that provides in-depth information regarding its isolation and bioactivity profile (Goel et al. 2023). The preference for carbon source and carbohydrate utilization of the isolate has been studied. It was discovered that monosaccharides were the preferred source for the growth of cell biomass (Supplementary Table S1). However, it is well-documented in the literature that complex carbon sources often stimulate the production of secondary metabolites. Moreover, studies have shown that starch is the most effective substrate for the production of antibiotics (Jonsbu et al. 2002). Hence, the carbon source utilization profile of *Nocardiopsis* sp. could be instrumental in enhancing the production rate of secondary bioactive compounds.

Our previous study conducted by Goel et al. in 2023 delved into the metabolic profile of *N. lucentensis* EMB25. We discovered a range of intriguing compounds that have the potential to inhibit and eliminate mature biofilms of *P. aeruginosa.* The BRIG tool shows that *N. lucentensis* EMB25 is closely related to *Nocardiopsis dassonvillei* NOCA502F based on BLAST search, which finds local regions of similarity and can be filtered to minimum percentage identity or E-cut value. The secondary metabolites profile revealed more BGCs in *N. lucentensis* EMB25 than *Nocardiopsis dassonvillei* NOCA502F, and three metabolites ectoine, isorenieratene and streptamidine are produced by both *Nocardiopsis* strains. Streptamidine is an amidine-containing peptide first reported to be produced by *Streptomyces albidoflavus* J1074. The biological significance of streptamidine remains unknown. However, the presence of streptamidine and streptamidine-like BGCs implies the role in the development and signalling of organisms among various terrestrial actinobacteria (Russell et al. 2021). Here, we have identified the streptamidine BGC presence in rare actinobacteria, *Nocardiopsis* species.

Table 2 Overview of 24 secondary metabolic biosynthesis gene clusters of *N. lucentensis* EMB25 detected by antiSMASH 2.0 server

	Type	Gene cluster		Most similar known cluster in reference strain		Similarity (%)	Accession number
		From	To				
1	T1PKS	171,435	228,402	J1-001-2	*Nocardiopsis dassonvillei* strain HZNU_N_1 chromosome	34	**CP022434.1**
2	T1PKS	467,314	530,232	Neocarzinostatin	*Haloechinothrix aidingensis* strain YIM 98,757 Scaffold2	50	JACCKD010000002.1
3	CDPS	941,349	962,056		*Streptomyces venezuelae* strain ATCC 14,584		CP029192
4	Terpene	986,160	1,011,268	Isorenieratene	*Streptomyces* sp. WMMB 322 WMMB 322 09	100	LIPX01000009
5	Terpene	1,252,845	1,273,172	2-methylisoborneol	*Nocardiopsis* sp. CNT312 B042DRAFT	5	AZXF01000011
6	Lanthipeptide class-iii	1,478,583	1,501,246	SapB	*Streptomyces radiopugnans* strain CGMCC	100	FOET01000001
7	Lanthipeptide class-iv	1,747,648	1,770,236	Siamycin, mathermycin, duramycin	*Kribbella* soli strain VKM Ac-2540	12	SHKR01000011
8	T1PKS	1,887,902	1,940,467	ML-449, neocarzilin A/neocarzilin B, jerangolid A/jerangolid D	*Nocardiopsis* sp. TSRI0078	28	LWLB01000036
9	RiPP-like	1,940,778	1,950,116	Streptamidine	*Streptomyces* sp. F-7 isolate *Streptomyces* sp	16	FKJH01000030
10	Lanthipeptide-class-i	1,958,49	1,982,355		*Nocardiopsis* sp. CNT312 B042DRAFT		AZXF01000017
11	NI-siderophore	2,168,128	2,181,814	nonactin/monactin/dinactin/trinactin/ tetranactin	*Nonomuraea indica* strain DRQ-2	33	KZ559467
12	Lanthipeptide-class-i	3,179,448	3,202,551	Cyphomycin	*Micromonospora haikouensis* strain DSM 45,626	2	FMCW01000048
13	Butyrolactone	3,329,801	3,340,886	scopranone A/scopranone B/scopranone C	*Nocardiopsis dassonvillei* strain NOCA502F	8	CP017965
14	Lanthipeptide-class-i	3,469,891	3,495,021		*Frankia casuarinae*		007777
15	NRPS	3,552,246	3,598,097	azetidomonamide A/azetidomonamide B	*Streptomyces* sp. CB04723 chromosome	16	CP058556

Table 2 (continued)

	Type	Gene cluster		Most similar known cluster in reference strain		Similarity (%)	Accession number
		From	To				
16	NRPS, T1PKS	3,600,982	3,650,623	frontalamide B/frontalamide, 10-epi-HSAF, 10-epi-3-deOH-HSAF, 10-epi-maltophilin, 10-epi-xanthobaccin C, 10-epi-hydroxymaltophilin, 10-epi-FI-2	*Lysobacter capsici* strain KNU-14 chromosome	50	CP011130
17	terpene	4,230,275	4,251,993				
18	LAP, thiopeptide	4,692,160	4,722,368	foxicin A/foxicin B/foxicin C/foxicin	*Nocardiopsis alba* strain TP-A0876	4	BAZE01000010
19	NRPS-like, T1PKS	5,192,713	5,241,519	Thiolactomycin, thiotetroamide, ebelactone A/ebelactone B	*Actinomadura* sp. RB29	15	MTBP01000003
20	Melanin	5,408,291 -	5,418,617	4-hydroxy-3-nitrosobenzamide, grixazone A, ferroverdin/bagremycin	*Streptomyces galilaeus* strain ATCC 14,969	47	CP023703
21	Ectoine	5,724,512	5,734,549	Enteromycin	*Nocardiopsis* sp. CNT312 B042DRAFT	8	AZXF01000005
22	T1PKS	5,760,661	5,840,765	Rosamicin, carrimycin	*Streptomyces noursei* ATCC 11,455	46	CP011533
23	RiPP-like	6,101,465	6,111,767		*Nocardiopsis dassonvillei* strain NOCA502F		CP017965
24	Ectoine	6,149,627	6,160,043	Ectoine	*Nocardiopsis dassonvillei* strain HZNU N 1	75	CP022434

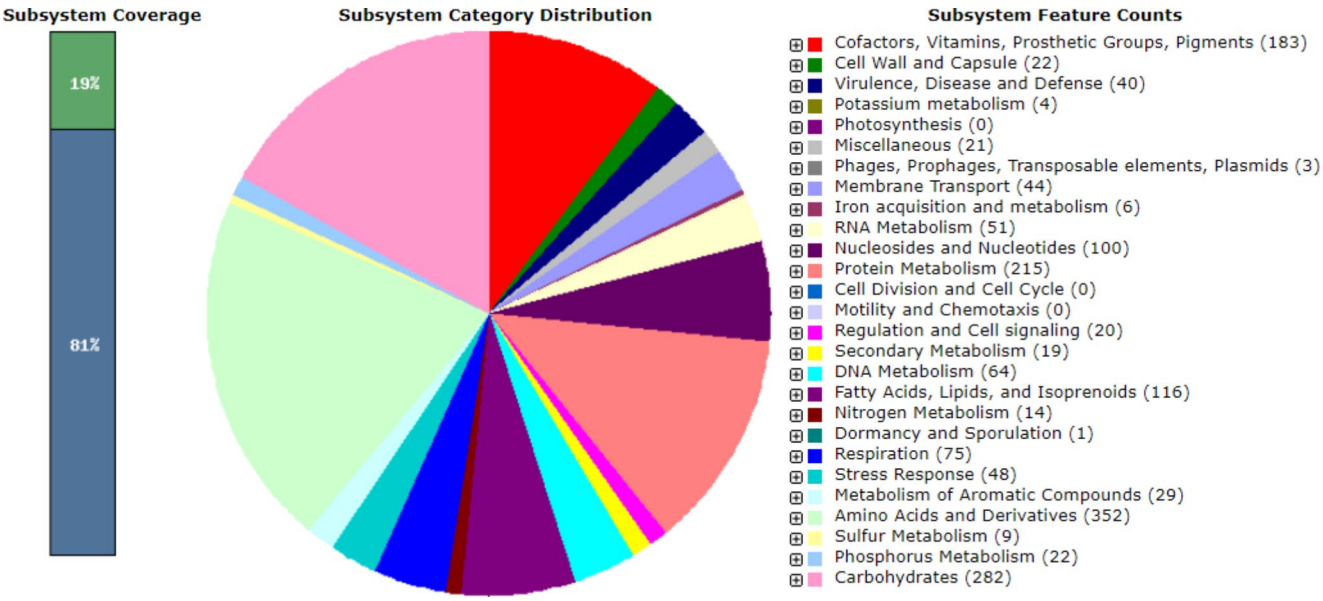


Fig. 4 Distribution of *N. lucentensis* EMB25 genes into subsystem categories. The numbers in parentheses show the counts of genes with specific functions

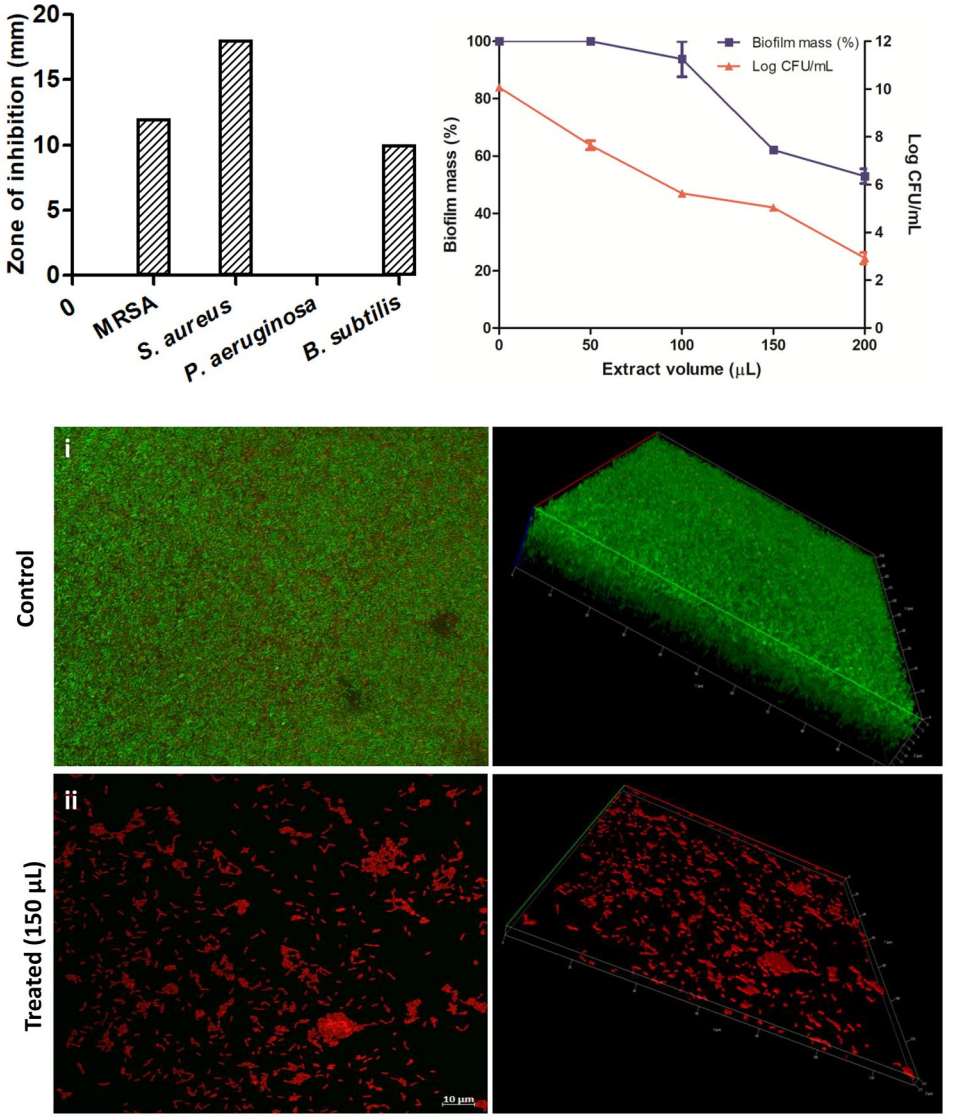


Fig. 5 **A**) The antibacterial activity of *N. lucentensis* EMB25 using agar-plug assay **B**) The effect of *N. lucentensis* EMB25 extract on preformed biofilm of *P. aeruginosa* PseA and cell count **C**) Confocal microscopic images of *P. aeruginosa* PseA (i) control, (ii) post-treatment with the extract

Microorganisms develop suitable solutes like ectoines to survive in environments with wildly varying osmolarities. Ectoines shield the DNA from ionising radiation, aid in lipid bilayer stabilization, shield protein function from a variety of stresses, and protect the bacterial cell from high temperatures (Goel et al. 2022; Salvador et al. 2018). However, based on the orthoANI values, the query genome is related to *N. arvandica* DSM4527. Among various overall genome relatedness indices (OGRI) algorithms developed, orthoANI is the most widely used for comparing the genomes and is most accurate. The orthoANI values obtained clearly indicate that *N. lucentensis* EMB25 has orthoANI values less than 85% of other closely related *Nocardiopsis* species inferring the difference and how closely related to the existing genome. Both *N. lucentensis* EMB25 and *N. arvandica* DSM4527 produces Lanthipeptide class i and LAPs inferring from the results obtained after BAGEL4 analysis (Supplementary Table S3). Lanthipeptides are post-translationally modified peptides which has antimicrobial properties and can be used as preservatives, probiotics, additives in cosmetics, and prophylactics. Class-i lanthipeptides are synthesized by two different enzymes: LanB (dehydratase enzyme) and LanC (cyclase enzyme). *N. arvandica* DSM4527 contains both domains whereas *N. lucentensis* EMB25 has only dehydratase domain. Lanthipeptides are post-translationally modified peptides which has antimicrobial properties and can be used as preservatives, probiotics, additives in cosmetics, and prophylactics (Bothwell et al. 2021).

In this study, we aim to uncover the genetic signatures of enzymes in biosynthetic pathways through genome mining. Specifically, our focus is on identifying and predicting the genes responsible for producing bioactive compounds in the marine rare actinobacteria *N. lucentencis* EMB25. The genetic basis behind production of bioactive compounds

opens the door to further discovery and provides an unparalleled opportunity to thoroughly examine the diversity and inner workings of life (Bauman et al. 2021). Furthermore, the *Nocardiopsis* genus is a valuable natural resource that merits exploration for the production of novel bioactive compounds with diverse structures and significant pharmaceutical applications (Bennur et al. 2015). The rich biosynthetic potential of this strain could be instrumental to predict promising molecules which could not be synthesized in laboratory settings. Additionally, there is a lack of studies on whole genome sequencing for this genus, particularly for this specific species. It is crucial to shift the research community's attention toward the potential utility of this genus for drug discovery, particularly in the fight against drug-resistant bacteria. This is essential due to the increasing threat of antimicrobial resistance, which is slowly becoming a silent pandemic.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11274-023-03738-6.

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Author contributions Nikky Goel: Conceptualization, Methodology, Formal analysis, writing original draft; Saniya Zaidi: Methodology, Formal analysis, writing; Sunil K. Khare: Conceptualization, Formal analysis, Supervision, Review MS.

Data Availability The bacterial genome of *N. lucentensis* EMB25 has been submitted to NCBI database with accession number: JAMTDN000000000, Bioproject: PRJNA729595 and Biosample: SAMN19135856.

Declarations

Competing interests The authors declare no competing interests.

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