

Complete genome sequence of *Pseudomonas* sp. HT11 isolated from broad bean (*Vicia faba* L.)

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Abstract

Bacterial strain HT11 was isolated from broad bean (*Vicia faba* L.) and found to have strong antifungal activity against the fungus *Botrytis fabiopsis* (which causes red spot of broad bean). To better understand the secondary metabolites of the HT11 strain, its complete genome was sequenced and analyzed. It contained a single circular chromosome, with a length of 6,335,588 bp. The 16S rRNA gene comparison and the average nucleotide identity (ANI) analysis confirmed that the HT11 strain is a new *Pseudomonas* strain. The complete genome encoded 5,366 predicted open reading frames (ORFs), 66 tRNA genes, and 16 rRNA genes. The total length of the annotated genes accounted for 82.93% (5,254,103/6,335,588 bp) of the complete genome. The predicted functional ORFs were grouped into 24 Clusters of Orthologous Groups of proteins (COG) categories. Fourteen gene clusters were detected in the genome, which were involved in the synthesis of pyochelin, pyocyanin, viscosin, and tolaasin I/tolaasin F. There were also three gene clusters involved in the synthesis of unknown metabolites. These results lay the foundation for further research on the interactions between *Pseudomonas* sp. HT11 and the pathogenic fungus *Botrytis fabiopsis*.

Introduction

Pseudomonas is a member of the genetically and phenotypically diverse class Gammaproteobacteria. *Pseudomonas* is an extremely diverse genus, with members found in a wide range of environments, including soil, water, air, plants, animals, and clinical materials.

Plant-associated *Pseudomonas* strains exhibit diverse metabolic capabilities, ensuring their adaptability and competitiveness in rhizomes and root layers, where they form symbiotic relationships, such as mutualism, commensalism, or parasitism, with their hosts (Clarke.1982 ;Silby et al. 2011). The beneficial effects of *Pseudomonas* on plants include growth promotion (e.g., by increasing nutrient absorption and root growth) and improved resilience to abiotic and biotic stresses (e.g., by influencing plant hormone levels) (Kuhl-Nagel et al .2022; Li et al .2022; Al-Enazi et al .2022). In particular, *Pseudomonas* can produce secondary metabolites play important roles in the activity of antagonistic bacteria against plant pathogens (such as cyclic lipopeptides, phenazines, and other antifungal compounds; siderophores; and bacterial secretion systems and associated toxins and effectors) play important roles in the activity of antagonistic bacteria against plant pathogens (De Vrieze et al .2020)

With the rapid development of computer technology and bioinformatics technology, high-throughput sequencing technology has been widely used in microbial genomes, enabling people to analyze microbial genomic information accurately and efficiently, and thus better explore the functions and mechanisms of microbial action (Zhou et al .2018; Rabbee et al. 2020). In recent years, researchers have used whole genome sequencing technology to study the biological characteristics of different *pseudomonas* strains, mine genomic information of strains, and predict the function of strains, which is of great significance for in-depth mining of bacteriocin synthesis gene clusters, identification of

bacteriocin specific genes, and functional genomics of bacteriocin (Joshi and Chitanand .2020; Zhou et al .2022; Lin et al .2017).

In this study, strain HT11, which was isolated from broad bean (*Vicia faba* L.) leaves and exhibited a strong biocontrol effect against the plant pathogenic fungus *Botrytis fabiopsis*, was studied. Antifungal activity assays showed that HT11 effectively inhibited *Botrytis fabiopsis* growth. The complete genome of HT11 was obtained by whole-genome sequencing. Using 16S rRNA sequencing and average nucleotide identity (ANI) analysis, HT11's taxonomic status as a *Pseudomonas* strain was accurately identified. Using the COG, KEGG, and other databases, the genome and secondary metabolite information of HT11 was analyzed in detail. AntiSMASH was used to analyze the a biosynthetic gene clusters of *Pseudomonas* sp. HT11, which provides a foundation for improved development of its application prospects.

Materials and Methods

HT11 strain and antifungal activity assay

The HT11 strain was previously isolated from broad bean (*Vicia faba* L.) leaves. It was found to have strong antifungal activity against the plant pathogenic fungus *Botrytis fabiopsis* (which causes red spot of broad bean) based on an antifungal activity assay. Briefly, a *Botrytis fabiopsis* mycelium block was placed in the center of a PDA plate. Next, 100 µl HT11 suspension was added around the mycelium block. The plate was placed in an incubator at 28°C for 5 days, and the size of each antifungal zone was measured. Inhibition rate = (inhibition zone diameter/colony diameter)×100%.

Genomic DNA extraction

Pseudomonas strain HT11 was cultured for 24 h, the bacteria were collected by centrifugation, and the DNA of the strain was extracted by bacterial Genomic DNA Purification Kit (Promega, Madison, WI, USA). The purified genomic DNA was quantified using a TBS-380 fluorometer (Turner BioSystems Inc., Sunnyvale, CA, USA). Next, high-quality DNA (optical density at 260 nm/optical density at 280 nm [OD₂₆₀/OD₂₈₀]: 1.8–2.0; >20 µg) was used for further analysis.

Whole genome sequencing

The sequencing was commissioned by Shanghai Meiji Biotechnology Co., LTD. After the samples passed the quality inspection, the second-generation sequencing technology and the third-generation high-throughput sequencing platform were used to complete the sequencing analysis, and the joint removal of the original data and low-quality sequences were optimized by quality statistical software. The complete genome sequence of HT11 has been deposited in GenBank under the accession number SUB14571263 (chromosome).

Genome assembly

Based on the third-generation sequencing data, Unicycler v0.4.8 was used for assembly. During the assembly process, sequence correction was performed using Pilon(version 1.22) to obtain the preliminary assembly results. SOAPdenovo2 was used to further correct the assembly results to obtain the final assembly results (Wick et al. 2016; Luo et al 2012).

Phylogenetic tree based on 16S rRNA gene

MEGA X (Kumar et al. 2018) was used to construct a neighbor-joining phylogenetic tree (with 1000 replications), based on the 16S rRNA gene, to determine the phylogenetic relationships of HT11 with closely related strains.

Genome annotation

Glimmer (Delcher et al. 2007) was used for coding sequence (CDS) prediction, tRNA-scan-SE (Borodovsky and Mcininch. 1993) was used for tRNA prediction, and Barrnap was used for rRNA prediction. The predicted CDSs were annotated using the NR, Swiss-Prot, Pfam, GO, COG, and KEGG databases using sequence alignment tools such as BLAST, Diamond, and HMMER. Briefly, the query proteins were aligned with the databases and the annotations of the best-matched proteins (e-value < 10^{-5}) were then obtained for annotation of the query proteins.

Analysis of secondary metabolite synthesis gene cluster

The online software AntiSMASH (Blin et al. 2021) was used to predict the secondary metabolite synthesis genome cluster in strain HT11.

Results

Antifungal activity assay

As shown in Fig. 1, HT11 had a good inhibitory effect against the fungus *Botrytis fabiopsis*, with an inhibitory rate of 81.08%.

Comparative genomic analysis

The isolated bacterial strain HT11 was closely related to *Pseudomonas* sp. LBUM920 (GCF_003852315.1), with an 16S rRNA gene sequence similarity of 99.93% (Fig. 2). Furthermore, the ANI value was highest for *Pseudomonas* sp. LBUM920 (96.55%). These results suggest that the HT11 strain belonged to the genus *Pseudomonas*.

Genome features

Pseudomonas sp. HT11 only had a single circular chromosome (Fig. 3a) and no plasmids. The circular genome was 6,335,588 bp in length, with a G+C content of 60.61%. It encoded 5,366 predicted open reading frames (ORFs), 66 tRNA genes, and 16 rRNA genes (5 16S rRNA genes, 5 23S rRNA genes, and 6

5S rRNA genes). The complete genome contained eight gene islands, four prophages, and one CRISPR-Cas system.

Functional prediction

The total length of the annotated genes accounted for 82.93% (5,254,103/6,335,588 bp) of the complete genome. The predicted functional ORFs were grouped into 24 COG categories. These categories involved storage and processing (A, 1 ORF; J, 282 ORFs; K, 511 ORFs; L, 162 ORFs; R, 449 ORFs; X, 86 ORFs), metabolism (C, 248 ORFs; E, 580 ORFs; F, 120 ORFs; G, 321 ORFs; H,286 ORFs, I, 277 ORFs; P, 317 ORFs; Q, 105 ORFs), cellular processes and signaling (D, 58 ORFs; M, 325 ORFs; N, 97 ORFs; O, 208 ORFs; T, 420 ORFs; U, 130 ORFs; V, 122 ORFs; W, 52 ORFs; Z, 2 ORF), and unknown functions (S, 207 ORFs) (Fig. 3b). Additionally, the predicted functional ORFs were categorized into six KEGG level 1 pathways, comprising cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organismal systems (Fig. 4).

Analysis of secondary metabolites

The antiSMASH results indicated that the HT11 genome harbored 14 putative biosynthetic gene clusters (BGCs) that might be responsible for secondary metabolite production. Of these, 11 exhibited similarity to known BGCs (Table 1). In particular, there was a phenazine BGC (shown in row 5 of Table 1) and a non-ribosomal peptide synthase (NRPS) BGC (shown in row 6 of Table 1) that exhibited 100% similarity to a known pyochelin BGC and a known pyocyanin BGC, respectively, from *Pseudomonas aeruginosa* PAO1 (Mavrodi et al. 2001; Reimmann et al. 2001). Additionally, a putative NRPS BGC (shown in row 4 of Table 1) was 75% similar to a known viscosin BGC from *Pseudomonas fluorescens* SBW25 (Braun et al .2001). Furthermore, a putative NRPS BGC (shown in row 10 of Table 1) was 70% similar to a known tolaasin I/tolaasin F BGC from *Pseudomonas costantinii* (Scherlach et al. 2013; Jo et al. 2001; Cho et al. 2007). As the remaining ten putative BGCs exhibited low (5–45%) or no similarity to known BGCs (Table 1), it is likely that *Pseudomonas* sp. HT11 can synthesize novel natural products. Further study of these putative BGCs and the bioactive compounds synthesized by them will help us to understand the ecological role that *Pseudomonas* sp. HT11 plays in plants and to develop its applications accordingly.

Table 1 Putative biosynthetic gene clusters (BGCs) in the HT11 genome predicted by antiSMASH

Cluster number	Type	Similar cluster	MIBiG accession no.	Similarity (%)	No. of genes
1	NRPS-like	ambactin	BGC0001131	25	24
2	arylpolyene	APE Vf	BGC0000837	45	37
3	RiPP-like	ND	ND	-	11
4	NRPS	viscosin	BGC0001312	75	32
5	NRPS	pyochelin	BGC0000412	100	33
6	phenazine	pyocyanin	BGC0000936	100	23
7	thiopeptide	lipopolysaccharide	BGC0000774	5	26
8	NRPS	pyoverdin	BGC0000413	11	34
9	betalactone	fengycin	BGC0001095	13	17
10	NRPS	tolaasin I/tolaasin F	BGC0000447	70	33
11	NAGGN	ND	ND	-	9
12	NRPS	pyoverdin	BGC0000413	10	41
13	redox-cofactor	lankacidin C	BGC0001100	13	16
14	RiPP-like	ND	ND	-	9

ND, no defined clusters are similar; NAGGN, N-acetylglutaminyglutamine amide; NRPS, non-ribosomal peptide synthase; MIBiG, Minimum Information about a Biosynthetic Gene cluster; RiPP-like, other unspecified ribosomally synthesized and post-transcriptionally modified peptide product.

Discussion

At present, the genera of the most frequently used biocontrol bacteria include *Pseudomonas*, *Bacillus*, and *Streptomyces*, with research mainly focusing on *Pseudomonas* and *Bacillus*. *Pseudomonas* sp. HT11, which was isolated from a plant by our research team, is a bacterial strain with promising biocontrol application potential. To clarify its taxonomic status and further analyze its biocontrol application potential, this study involved whole-genome sequencing of HT11, combined with bioinformatics analyses to determine its taxonomic status and analyze its disease resistance effects.

HT11 harbors a rich variety of biosynthetic gene clusters (BGCs) They are involved in the synthesis of secondary metabolites such as pyochelin and pyocyanin, and secondary metabolites related to viscosin, tolaasin I/tolaasin F. Some of the bacterial secondary metabolites inhibit the growth of pathogenic fungi (Alsohim et al. 2014), and some (such as pyochelin and pyocyanin) also induce plant defense

mechanisms (Audenaert et al. 2020). In addition to biosynthetic gene clusters with known functions, HT11 harbors three with unknown functions, which indicates that it has promising application potential that should be developed.

Declarations

Author contributions HZ, LM, and WL wrote the manuscript with support from all authors. RT and XH revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest All the authors declare that they have no conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All the authors consented to publish the article.

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Figures

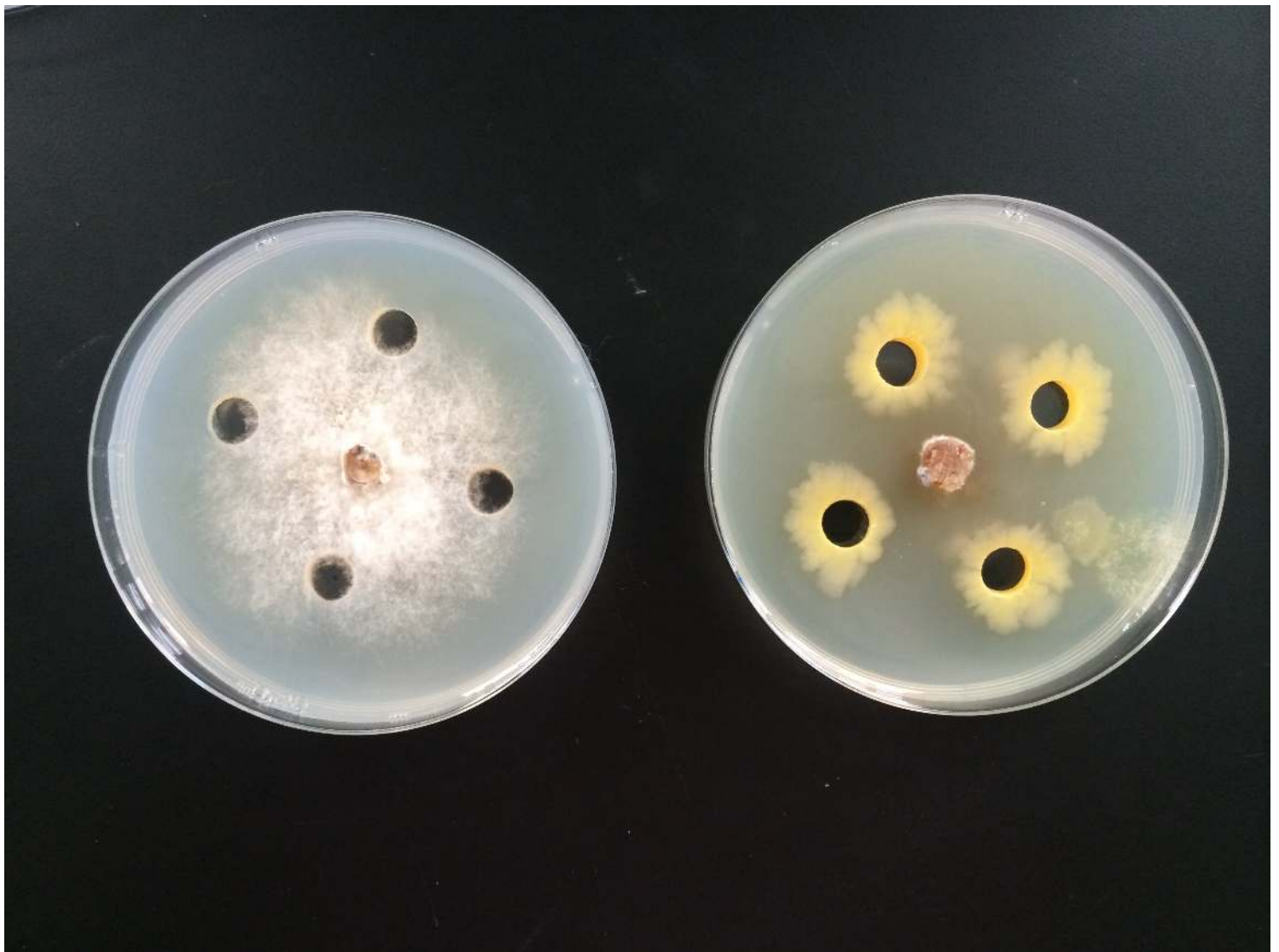


Figure 1

Inhibitory effect of HT11 against fungus *Botrytis fabiopsis*.

A:As a contrast; B:Bacteriostasis for Botryotinia fabae.

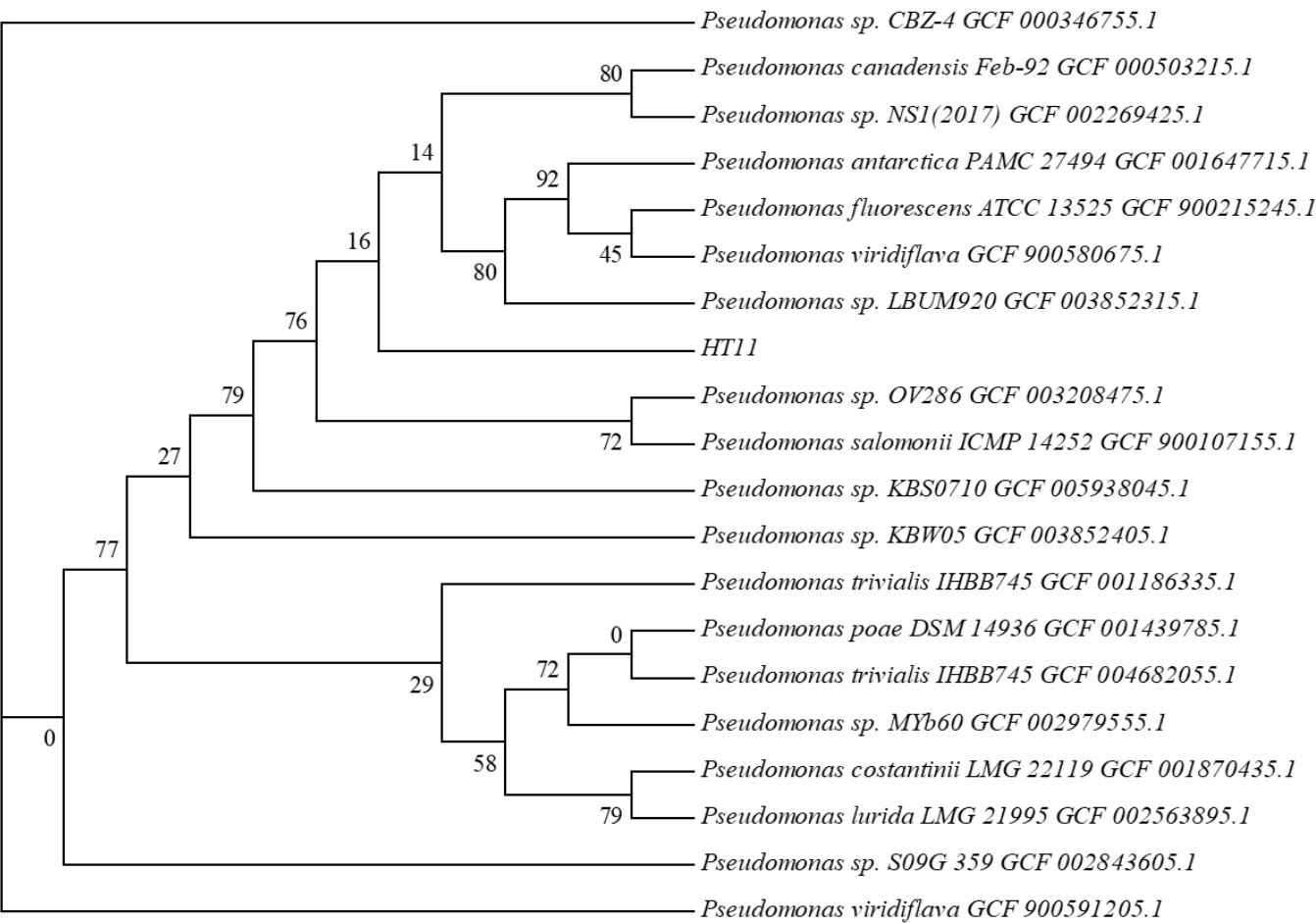
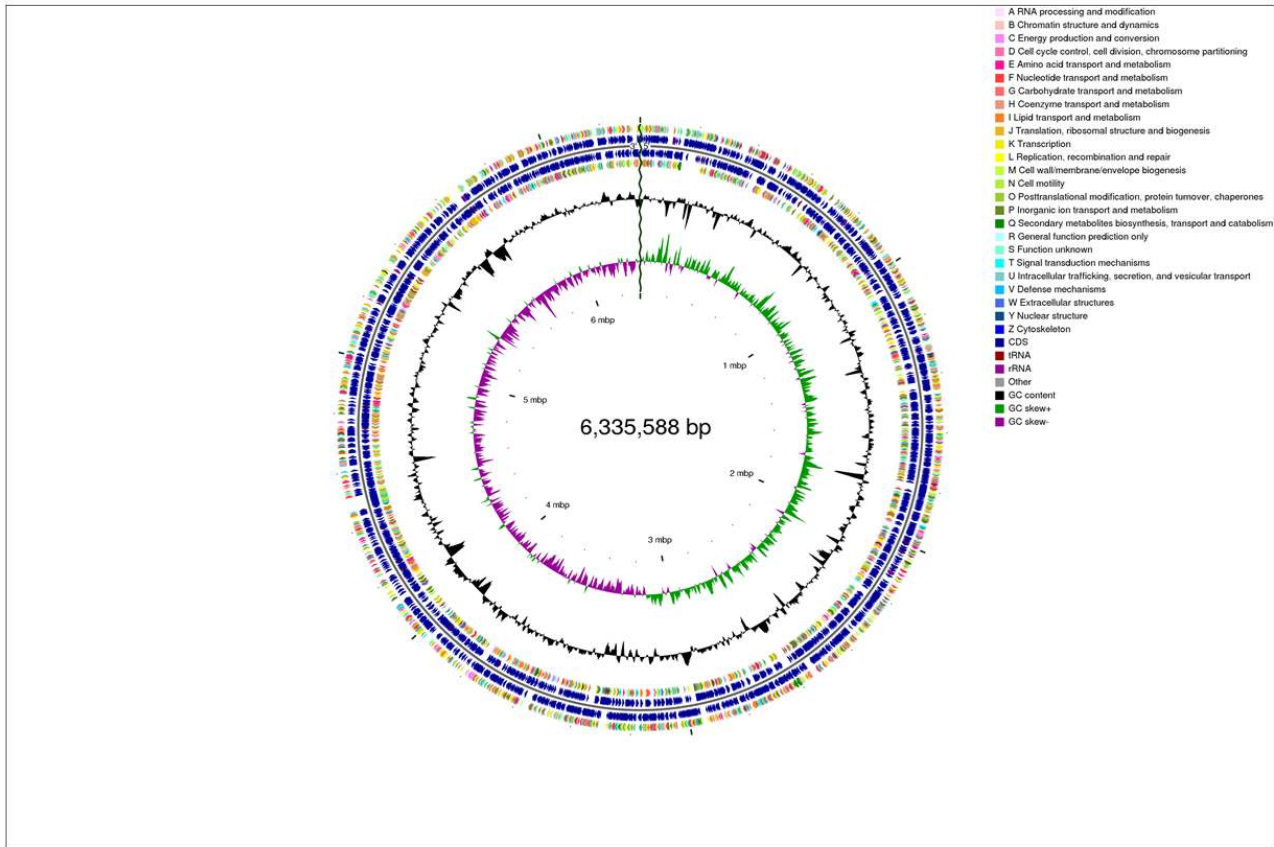


Figure 2

16S rRNA gene similarity between HT11 strain and the type strains of closely related species of the genus *Pseudomonas*.

a



b

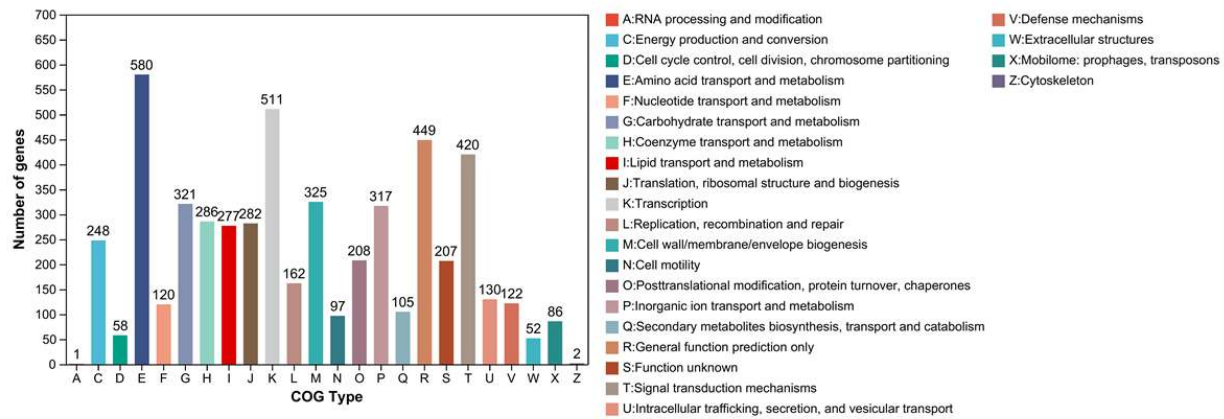


Figure 3

(a) Circular genome map of *Pseudomonas* sp. HT11. (b) COG functional annotation of the coding sequences (CDSs) of *Pseudomonas* sp. HT11.

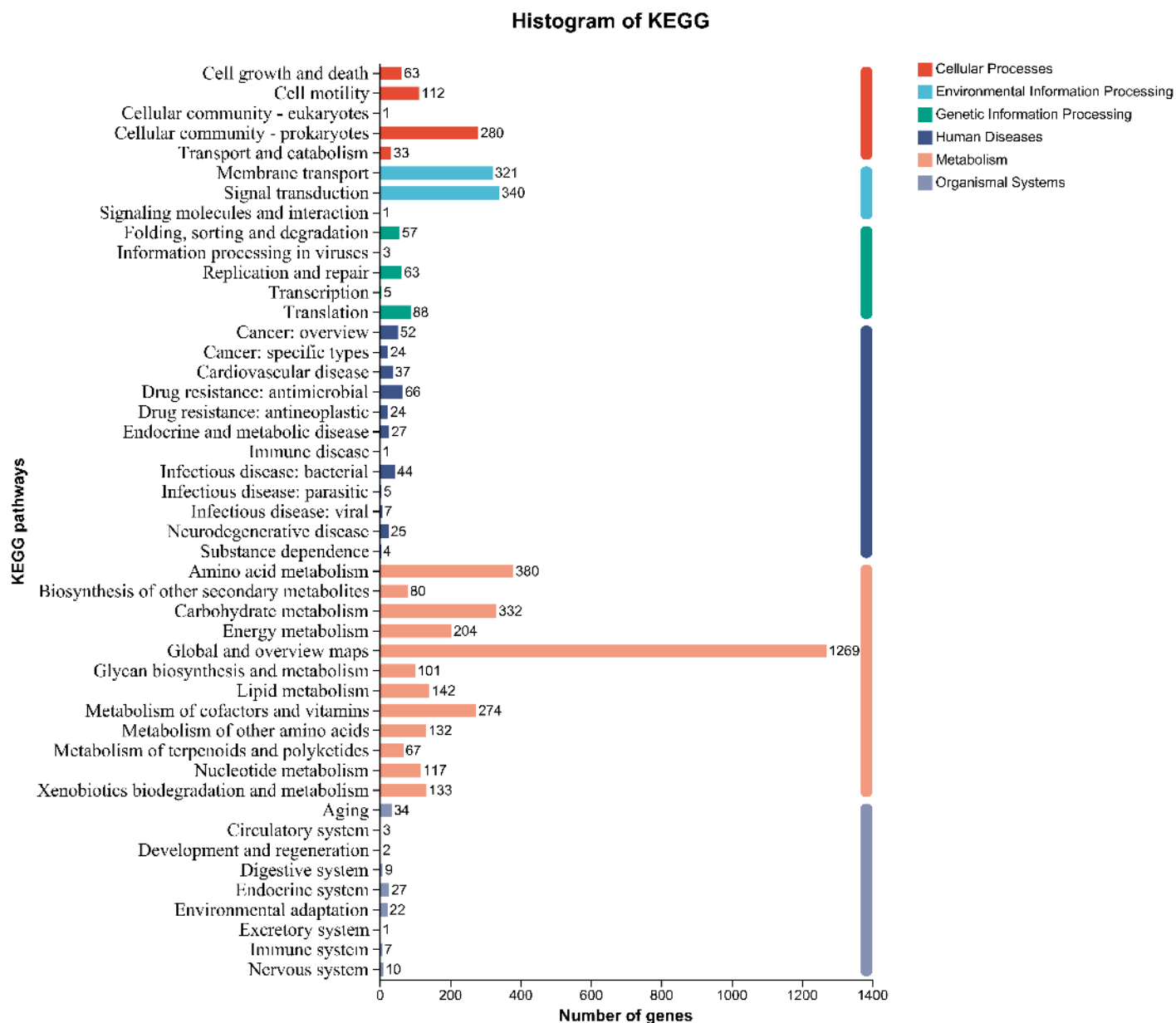


Figure 4

KEGG functional annotation of the coding sequences (CDSs) of *Pseudomonas* sp. HT11.