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A Comprehensive Genome Mining Analysis of Biosynthetic Gene Clusters in *Pseudomonas* sp. SXM-1

Pseudomonas sp.'deki Biyosentetik Gen Kümelerinin Detaylı Genom Madenciliği Analizi

Türk Denizcilik ve Deniz Bilimleri Dergisi

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ABSTRACT

Very resistant pathogenic microorganisms have been reported to current antibiotics in the last decade. Therefore, there is a great need to understand not only resistance metabolism but also secondary metabolites of pathogenic microorganisms. Genome mining tools have so far been improved to understand secondary metabolites from biosynthetic gene clusters. Microorganisms whose genomes and secondary metabolites are predicted by these tools are widely used in the pharmaceutical and industrial studies. *Pseudomonas* spp. are widely used in recombinant DNA technology to produce commercial products. Bioinformatics-based *in silico* tools significantly contribute to the discovery of new bioactive compounds for pharmacy and medicine. This study aims to conduct a comprehensive gene cluster analysis of the *Pseudomonas* sp. SXM-1 strain isolated from the coastal seawater of Xiamen Bay using antiSMASH (7.0.1). The accession number of *Pseudomonas* sp. SXM-1 strain was retrieved from NCBI. 14 regions were found, including non-ribosomal peptides metallophores (NRP-metallophore), nonribosomal peptide-synthetase (NRPS), NRPS-like, ribosomally synthesized and post-translationally modified peptide-like (RiPP-like), betalactone, nonribosomal peptide-synthetase (NRPS), ectoine and N-acetylglutaminylglutamine amide (NAGGN). Analysis of all 14 regions revealed secondary metabolites with potential applications in diverse fields. Microbiologists are strongly advised to conduct wet-lab experiments to validate the secondary metabolites discussed in this study.

Keywords: antiSMASH, biosynthetic gene cluster, genome, *Pseudomonas* sp. SXM-1

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ÖZET

Son on yılda mevcut antibiyotiklere karşı çok dirençli patojen mikroorganizmaların olduğu rapor edilmiştir. Bu nedenle patojenik mikroorganizmaların sadece direnç metabolizmasını değil aynı zamanda sekonder metabolitlerini de anlamaya büyük ihtiyaç vardır. Günümüze kadar biyosentetik gen kümelerindeki sekonder metabolitleri ortaya çıkarmaya yönelik genom madenciliği araçları geliştirilmiştir. Bu araçları kullanarak genomları ve sekonder metabolitleri tahmin edilen mikroorganizmalar, farmasötik ve endüstriyel çalışmalarda yaygın olarak kullanılmaktadır. *Pseudomonas* spp. ticari ürünler üretmek için rekombinant DNA teknolojisinde yaygın olarak kullanılmaktadır. Biyoenformatik tabanlı *in silico* araçları, eczacılık ve tıp için yeni biyoaktif bileşiklerin keşfedilmesine önemli ölçüde katkıda bulunmaktadır. Bu çalışma, AntiSMASH (7.0.1) kullanılarak Xiamen Körfezi'nin deniz suyundan izole edilen *Pseudomonas* sp. SXM-1 suşunun kapsamlı bir gen kümesi analizini yapmayı amaçlamaktadır. *Pseudomonas* sp.'nin erişim numarası NCBI'dan alınmıştır. Ribozomal olmayan peptitler metaloforlar (NRP-metalofor), ribozomal olmayan peptit sentetaz (NRPS), NRPS benzeri, ribozomal olarak sentezlenmiş ve translasyon sonrası modifiye edilmiş peptit benzeri (RiPP benzeri), betalakton, ribozomal olmayan peptit sentetaz (NRPS), ektoin ve N-asetilglutaminilglutamin amid (NAGGN) dahil olmak üzere 14 bölge bulunmuştur. 14 bölgenin genom analizi, farklı alanlarda potansiyel uygulamalara sahip sekonder metabolitleri ortaya çıkarmıştır. Mikrobiyologlara bu çalışmada tartışılan sekonder metabolitleri doğrulamak için laboratuvar deneyleri yapmaları şiddetle tavsiye edilir.

Anahtar sözcükler: antiSMASH, biyosentetik gen kümesi, genom, *Pseudomonas* sp. SXM-1

1. INTRODUCTION

The development of *in silico* tools for genome mining paves the way for improved prediction of secondary metabolites. Simple chemical substances known as antibiotics have the ability to specifically and selectively destroy infectious bacteria. Natural products, whether natural, synthetic, or semi-synthetic, have potential to treat infectious diseases in their original or modified forms. Macromolecules are also obtained from various microorganisms and plants (Arulprakasam and Dharumadurai, 2021). Fungal and bacterial secondary metabolism has potential pharmaceutical applications such as cholesterol-lowering drugs, antitumor drugs, and antibiotics (Keller, 2019; Ramírez-Rendon *et al*., 2022). *In silico* tools are good options for genome mining because it takes time and effort to find each set of genes experimentally. antiSMASH is one of these tools and provides *in silico* analysis related to secondary metabolite compound clusters as terpenes, polyketides, non-ribosomal peptides, siderophores and others (Medema *et al*., 2011). *Pseudomonas* genus is a non-fermentative, gram-negative, gamma proteobacteria (Ye *et al*., 2013). The widespread presence of these microorganisms is indicative of the diversity of secondary metabolites of fluorescent *Pseudomonas* spp. Guo *et al*. (2021) isolated *Pseudomonas* sp. SXM-1 from the seawater of Xiamen Bay (China). They fully characterized a siderophore using antiSMASH technology. However, a new version of antiSMASH (7.0.1) revealed novel biosynthetic gene clusters and related secondary metabolites. This study presents a comprehensive biosynthetic gene cluster analysis of *Pseudomonas* sp. SXM-1.

2. MATERIALS AND METHODS

The NCBI accession number of *Pseudomonas* sp. SXM-1 strain is CP038001.1. The antiSMASH tool is used to identify biosynthetic gene clusters or secondary metabolite biosynthesis gene clusters (Blin *et al*., 2021). The default parameters were used for the antiSMASH analysis with relaxed detection strictness.

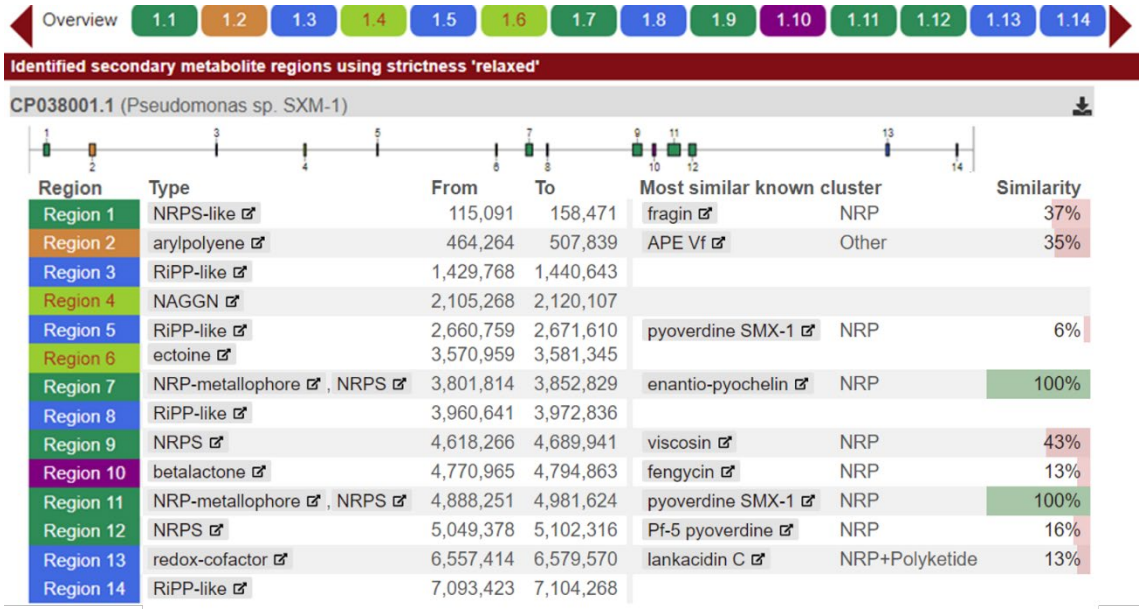
3. RESULTS

AntiSMASH analysis was used to determine secondary metabolites of *Pseudomonas* sp.

SXM-1 genome. Biosynthetic gene regions were identified as Non-ribosomal peptides metallophores (NRP-metallophore), nonribosomal peptide-synthetase (NRPS), NRPS-like, ribosomally synthesized and post-translationally modified peptide-like (RiPP-like), betalactone, nonribosomal peptide-synthetase (NRPS), ectoine and N-acetylglutaminylglutamine amide (NAGGN). NRPSs are modular mega enzymes that function via multiple covalent-linked domains. Adenylation (A), thiolation (T), and condensation (C) are minimal set for biosynthesis of non-ribosomal peptides. Adenylation results in hydrolysis of ATP. The C domain mediates the formation of peptide bonds in two adjacent modules. NRPS-like enzymes catalyze various reactions such as Dieckmann cyclization, reduction, and Claisen condensation (Shi *et al*., 2021). RiPPs are a large group of structurally diverse natural products (Ortega and van der Donk, 2016). Betalactone is a four-membered heterocyclic compound with a high ring strain, high electrophilicity, and good reactivity. Actinobacteria and fungi are responsible for a large portion of its natural products and many of them have powerful medicinal properties (Wang and Yao, 2022). Ectoine, as a highly water-retaining compound that stabilizes biomolecules and entire cells, can be used in scientific studies, cosmetology, and medicine (Reshetnikov *et al*, 2011).

According to Figure 1, 14 genomic regions were obtained from *Pseudomonas* sp. SXM-1 genome based on antiSMASH analysis.

In Figure 2, it is shown that region 1.1 includes NRPS-like gene proteins that have many functions in primary and secondary metabolism. It is located between 115,091 -158,471 nt. There are transcriptional regulator GcvA, acyl-CoA dehydrogenase gene, CoA transferase, TonB-dependent siderophore, sigma-70 family RNA polymerase sigma factor, aminobenzoate oxygenase, TauD/TfdA family dioxygenase, ABC transporter ATP-binding protein, ABC transporter permease, LysR family transcriptional regulator, MFS transporter, HlyD family secretion protein, efflux transporter outer membrane subunit and MarR family transcriptional regulator genes in this region.



Region	Type	From	To	Most similar known cluster		Similarity
Region 1	NRPS-like	115,091	158,471	fragin	NRP	37%
Region 2	arylpolyene	464,264	507,839	APE Vf	Other	35%
Region 3	RiPP-like	1,429,768	1,440,643			
Region 4	NAGGN	2,105,268	2,120,107			
Region 5	RiPP-like	2,660,759	2,671,610	pyoverdine SMX-1	NRP	6%
Region 6	ectoine	3,570,959	3,581,345			
Region 7	NRP-metallophore , NRPS	3,801,814	3,852,829	enantio-pyochelin	NRP	100%
Region 8	RiPP-like	3,960,641	3,972,836			
Region 9	NRPS	4,618,266	4,689,941	viscosin	NRP	43%
Region 10	betalactone	4,770,965	4,794,863	fengycin	NRP	13%
Region 11	NRP-metallophore , NRPS	4,888,251	4,981,624	pyoverdine SMX-1	NRP	100%
Region 12	NRPS	5,049,378	5,102,316	Pf-5 pyoverdine	NRP	16%
Region 13	redox-cofactor	6,557,414	6,579,570	lankacidin C	NRP+Polyketide	13%
Region 14	RiPP-like	7,093,423	7,104,268			

Figure 1. Biosynthetic gene clusters related to secondary metabolites in *Pseudomonas* sp. SXM-1 genome.

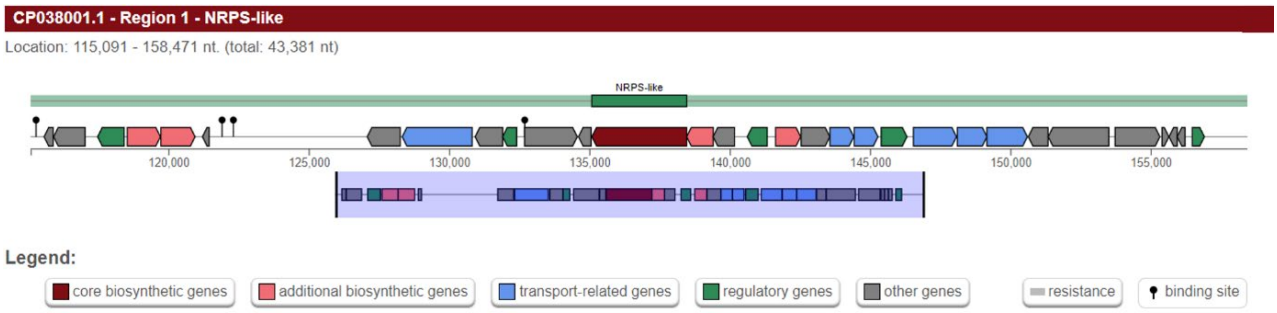


Figure 2. Nonribosomal peptide synthetases like (NRPS-like) gene clusters in *Pseudomonas* sp. SXM-1 genome.

Region 1.2 in the antiSMASH analysis revealed arylpolyene gene clusters (Figure 3). Aryl polyenes are polyunsaturated carboxylic acids (Johnston *et al*., 2021). These gene clusters are located between 464,264 - 507,839 nt. 3-dehydroquinate synthase, glutamate synthase small subunit, MFS transporter, LysR family transcriptional regulator, beta-ketoacyl-ACP synthase, 3-oxoacyl-ACP reductase FabG, beta-ketoacyl-[acyl-carrier-protein] synthase family protein, class I SAM-dependent methyltransferase, glycosyltransferase family 2 protein, AMP-binding protein, acyl carrier protein and 1-acyl-sn-glycerol-3-phosphate acyltransferase genes were also found.

In antiSMASH analysis, ribosomally synthesized and post-translationally modified peptide (RiPP) was detected in region 1.3. These regions were found between 1,429,768-1,440,643 nt (Figure 4). Short chain dehydrogenase, LysR family transcriptional regulator, DUF692 domain-containing protein, ABC transporter ATP-binding protein, high-affinity branched-chain amino acid ABC transporter permease LivM and high-affinity branched-chain amino acid ABC transporter permease LivH genes were also identified within the region 1.3.

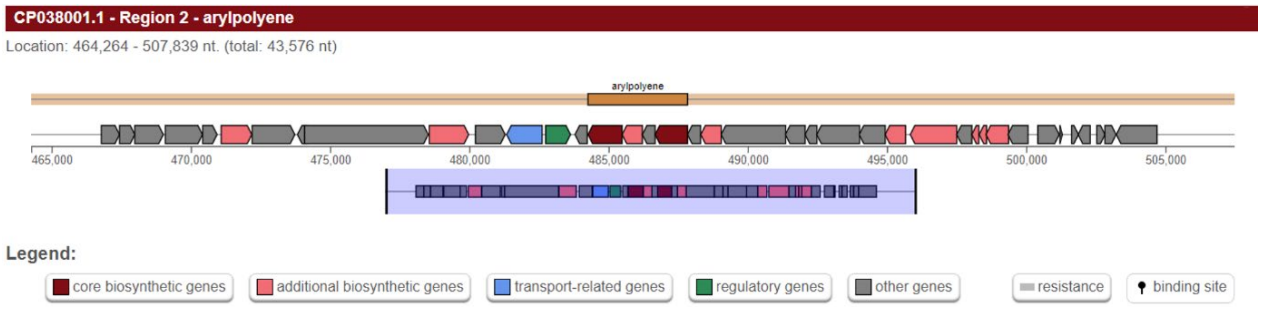


Figure 3. Arylpolyene gene region in *Pseudomonas* sp. SXM-1 genome.

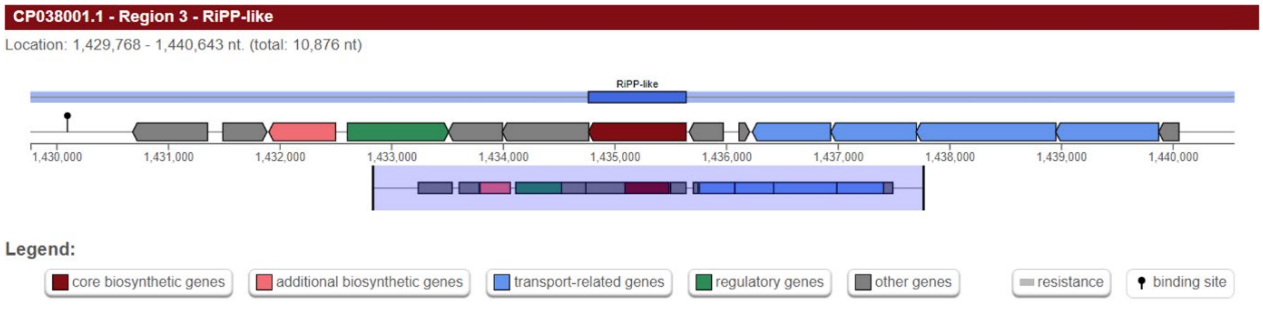


Figure 4. First ribosomally synthesized and post-translationally modified peptides like (RiPP-like) gene clusters in *Pseudomonas* sp. SXM-1 genome.

In Region 1.4, NAGGN related genes were identified (Figure 5). It is located between 2,105,268 - 2,120,107 nt. There are SDR family oxidoreductase, osmoprotectant NAGGN system M42 family peptidase, N-acetylglutaminylglutamine synthetase, N-acetylglutaminylglutamine amidotransferase and bifunctional tRNA (5-methylaminomethyl-2-thiouridine)(34)-methyltransferase MnmD/FAD-dependent 5-carboxymethylaminomethyl-2-thiouridine(34) oxidoreductase MnmC.

Interestingly, to note that we also observed a second region related to RiPP-like biosynthetic gene clusters within region 1.5 (Figure 6). Alpha/beta hydrolase, DUF692 domain-containing protein and MFS transporter genes were identified in this region. The position of region 1.5 is between 2,660,759 - 2,671,610 nt. Ectoine, a solute that prevents osmotic stress in highly saline environments, is located between 3,570,959 - 3,581,345 nt (Figure 7) (Reshetnikov *et al*., 2011). MFS transporter, ectoine synthase, response regulator, HAMP domain-containing protein and class A beta-lactamase-related serine hydrolase genes were identified in region 1.6.

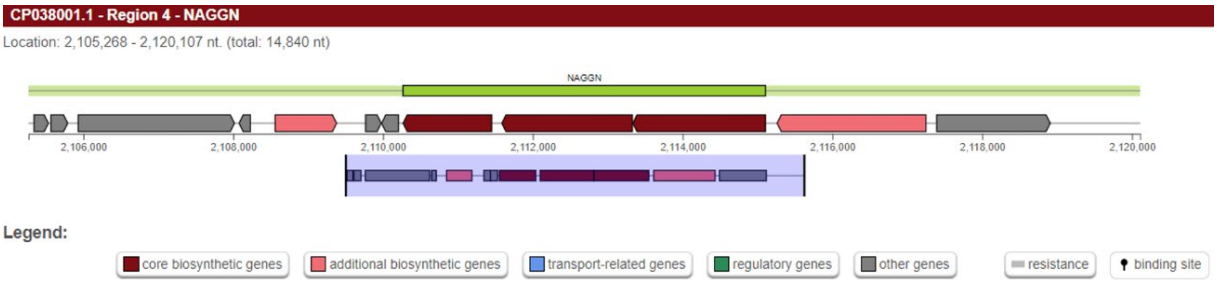


Figure 5. N-acetylglutamylglutamine amide (NAGGN) gene clusters in *Pseudomonas* sp. SXM-1 genome.

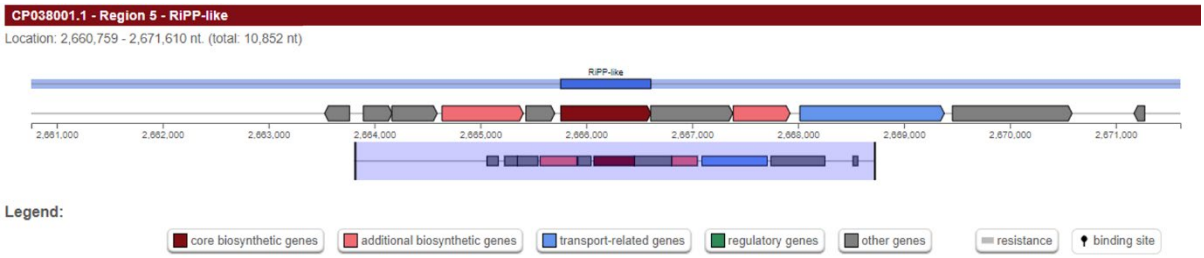


Figure 6. Second RiPP-like gene clusters in *Pseudomonas* sp. SXM-1 genome.

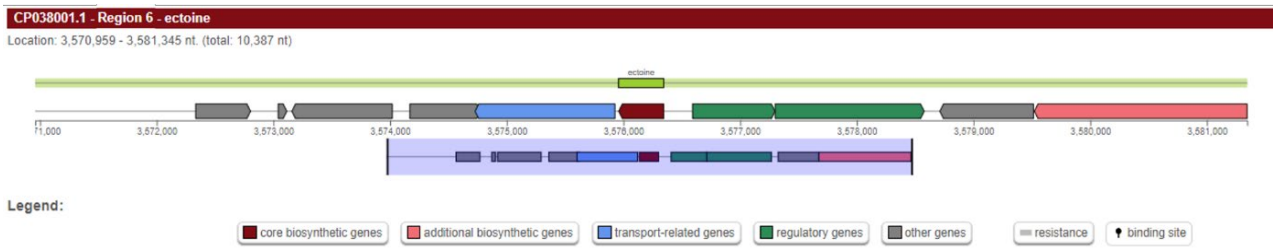


Figure 7. Ectoine gene clusters in *Pseudomonas* sp. SXM-1 genome.

It is shown that region 1.7 includes NRP-metallophore, NRPS genes that are synthesized by multidomain megaenzymes (Figure 8). These genes are located between 3,801,814 - 3,852,829 nt. Sugar ABC transporter substrate-binding protein, cupin domain-containing protein, efflux

RND transporter periplasmic adaptor subunit, efflux RND transporter permease subunit, TolC family protein, glutamate decarboxylase, isochorismate synthase, thioesterase, non-ribosomal peptide synthetase, amino acid adenylation domain-containing protein, ABC transporter ATP-binding protein, 2,3-dihydroxybenzoate-AMP ligase, AraC family transcriptional regulator, TonB-dependent siderophore receptor, iron ABC transporter permease, MFS transporter and TetR/AcrR family transcriptional regulator genes were also found.

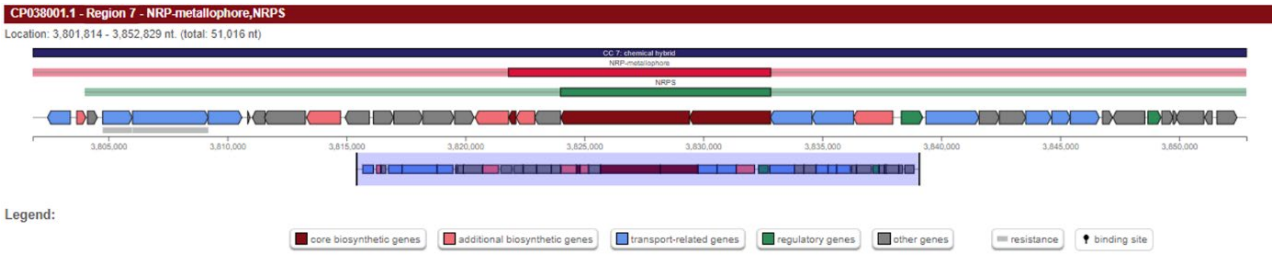


Figure 8. NRP-metallophore, NRPS gene clusters in *Pseudomonas* sp. SXM-1 genome.

In the antiSMASH analysis, a third RiPP-like gene was detected, located between 3,960,641 and 3,972,836 nt. There are HlyD family secretion protein, DHA2 family efflux MFS transporter permease subunit, efflux transporter outer membrane subunit, OsmC domain/YcaO domain-containing protein, alpha/beta hydrolase, HAD family phosphatase, LysR family transcriptional regulator, N-acetyl-gamma-glutamyl-phosphate reductase and epoxide hydrolase in the region 1.8 (Figure 9).

Figure 9. RiPP-like gene clusters in *Pseudomonas* sp. SXM-1 genome.

Figure 10 shows that region 1.9 includes the second NRPS that is located between 4,618,266 - 4,689,941 nt (Figure 10). Sigma-70 family RNA polymerase sigma factor, N-acetyltransferase, EamA family transporter, AraC family transcriptional regulator, GlxA family transcriptional regulator, MATE family efflux transporter, aliphatic amidase, Lrp/AsnC family transcriptional regulator, methionine gamma-lyase, MacB family efflux pump subunit, macrolide transporter subunit MacA, non-ribosomal peptide synthetase, amino acid adenylation domain-containing protein, efflux transporter outer membrane subunit, LysR family transcriptional regulator, glutathione S-transferase, glucose 1-dehydrogenase, L-idonate 5-dehydrogenase, ATP-binding cassette domain-containing protein, metallophosphoesterase and SDR family oxidoreductase were also found.

Figure 11 shows that 1.10 includes a betalactone that is located between 4,770,965 - 4,794,863 nt. Acyl-CoA dehydrogenase, LysR family transcriptional regulator, MerR family DNA-binding transcriptional regulator, hydroxymethylglutaryl-CoA lyase, AMP-binding protein, isovaleryl-CoA dehydrogenase, methylcrotonoyl-CoA carboxylase, gamma-carboxygeranoyl-CoA hydratase and acetyl/propionyl/methylcrotonyl-CoA carboxylase subunit alpha were also identified in the region 1.10 (Figure 11).

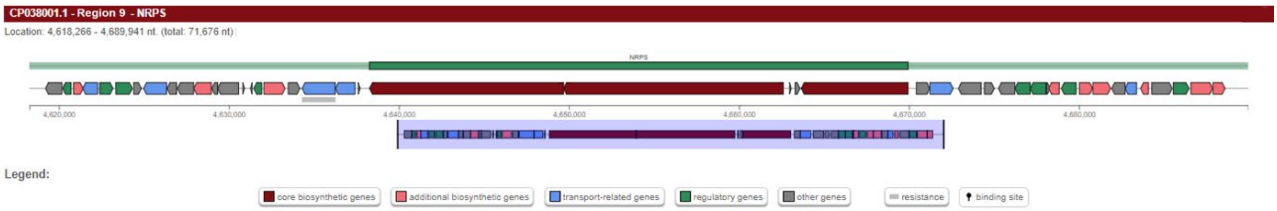


Figure 10. Second NRPS gene clusters in *Pseudomonas* sp. SXM-1 genome.

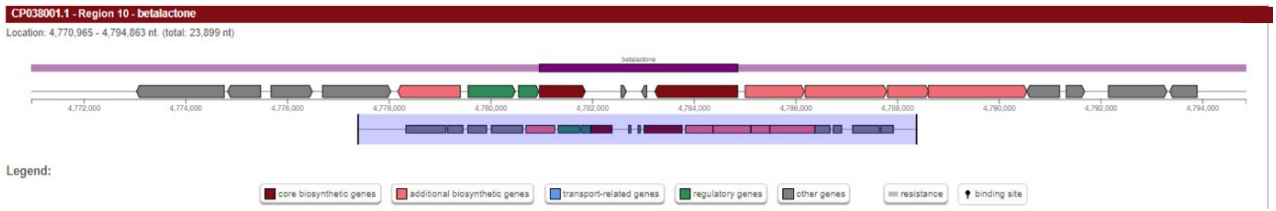


Figure 11. Betalactone genes in *Pseudomonas* sp. SXM-1 genome.

We also observed a second NRP-metallophore, NRPS biosynthetic gene clusters within the region 1.11 that is located between 4,888,251 - 4,981,624 nt (Figure 12). MBL fold metallo-hydrolase, Lrp/AsnC family transcriptional regulator, alpha-ketoacid dehydrogenase subunit beta, dihydrolipoyl dehydrogenase, ornithine cyclodeaminase, ABC transporter substrate-binding protein, ABC transporter permease subunit, histidine ABC transporter permease HisM, ATP-binding cassette domain-containing protein, ornithine monooxygenase, sigma-70 family RNA polymerase sigma factor, efflux RND transporter periplasmic adaptor subunit, MacB family efflux pump subunit, efflux transporter outer membrane subunit, PvdJ/PvdD/PvdP-like protein, aminotransferase class V-fold PLP-dependent enzyme, formylglycine-generating enzyme family protein, cyclic peptide export ABC transporter, TonB-dependent siderophore receptor, alpha/beta hydrolase, amino acid adenylation domain-containing protein, MFS transporter, TetR/AcrR family transcriptional regulator, DHA2 family efflux MFS transporter permease subunit, HlyD family secretion protein, TolC family protein, AraC family transcriptional regulator and SDR family oxidoreductase, GAF domain-containing protein, GlxA family transcriptional regulator and LysE family translocator were found.

In region 1.12, the third NRPS genes were obtained that are located between 5,049,378 - 5,102,316 nt (Figure 13). ABC transporter substrate-binding protein, metal ABC transporter ATP-binding protein, metal ABC transporter substrate-binding protein, MbtH family protein, aspartate aminotransferase family protein, two-component sensor histidine kinase, response regulator, thiol:disulfide interchange protein DsbG, amino acid adenylation domain-containing protein, thioesterase, RNA polymerase factor sigma-70, N-acetyltransferase, TetR/AcrR family transcriptional regulator, SDR family oxidoreductase, acyl-CoA carboxylase subunit beta, acyl-CoA dehydrogenase, enoyl-CoA hydratase/isomerase family protein, acetyl/propionyl/methylcrotonyl-CoA carboxylase subunit alpha, chemotaxis protein CheV, sensor histidine kinase and response regulator transcription factor were found in redox-cofactor gene clusters.

Figure 12. NRP-metallophore, NRPS gene clusters in *Pseudomonas* sp. SXM-1 genome.

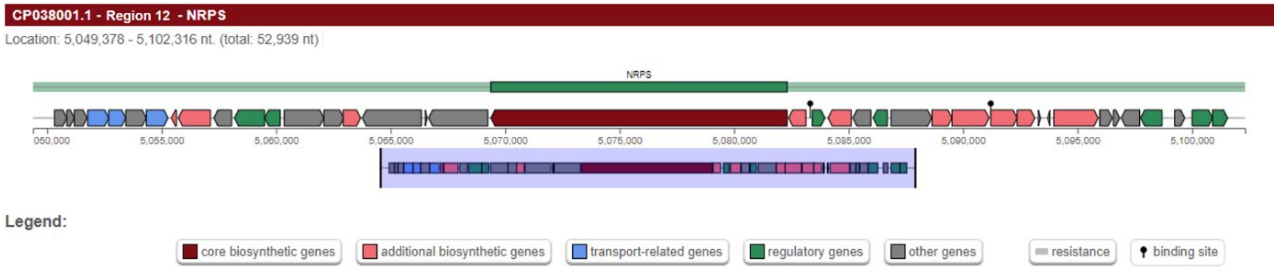


Figure 13. Third NRPS gene clusters in *Pseudomonas* sp. SXM-1 genome.

Figure 14 shows that region 1.13 includes redox-cofactor genes located between 6,557,414 - 6,579,570 nt (Figure 14). Lrp/AsnC family transcriptional regulator, carbon-nitrogen hydrolase family protein, pyrroloquinoline quinone biosynthesis protein PqqF, pyrroloquinoline-quinone synthase PqqC, pyrroloquinoline quinone biosynthesis peptide chaperone PqqD, pyrroloquinoline quinone biosynthesis protein PqqE, aminotransferase class III-fold pyridoxal phosphate-dependent enzyme, LysR family transcriptional regulator, acyl-CoA dehydrogenase and GGDEF domain-containing protein were found in this region.

According to Figure 15, RiPP-like is located between 7,093,423 - 7,104,268 nt. Methyltransferase domain-containing protein and DUF692 domain-containing protein were identified in this region.

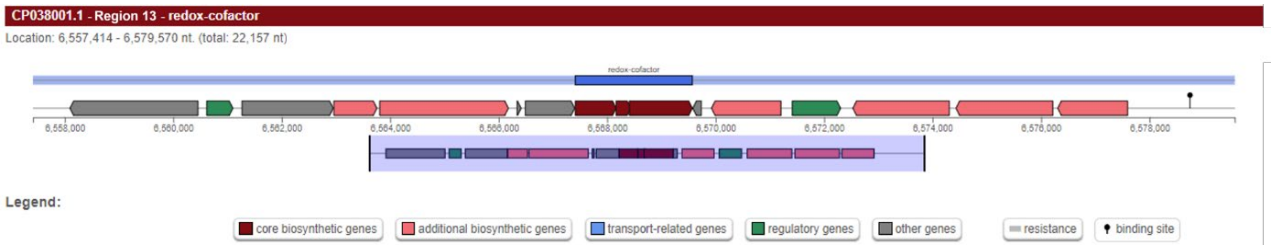


Figure 14. Redox-cofactor gene clusters in *Pseudomonas* sp. SXM-1 genome.

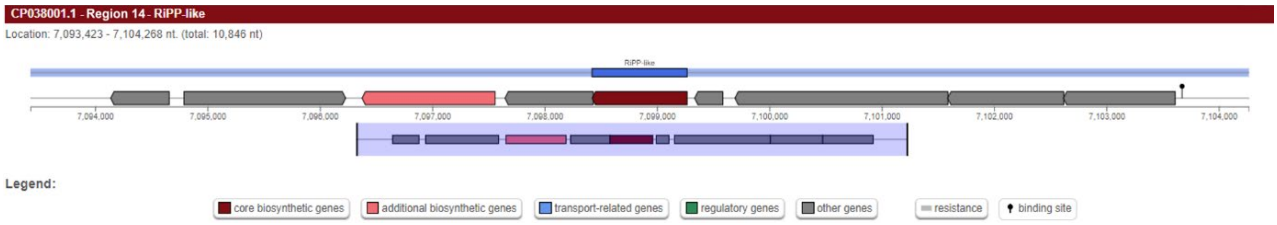


Figure 15. RiPP-like gene clusters in *Pseudomonas* sp. SXM-1 genome

4. DISCUSSIONS

Very resistant pathogenic microorganisms have been reported for the current antibiotics in the last decade. Therefore, there is a great need to understand not only resistance metabolism but also secondary metabolites of pathogenic microorganisms (Martens and Demain, 2017; Anand *et al*., 2020). The latter requires adaptations to new conditions and environments for survival. To be a dominant microorganism, they should synthesize novel secondary metabolites (Palazzotto and Weber, 2018). Recent studies have underlined the importance of the efficacy of novel secondary metabolites of pathogenic microorganisms in the war against other microorganisms under the same conditions (Tiwari *et al*., 2018; Zheng *et al*., 2019). Characterization of secondary metabolites requires wet-lab studies and instrumentation such as HPLC, NMR, FTIR, UV-VIS spectrophotometry and mass spectrometry (Kumar *et al*., 2021; Perruchon *et al*., 2021). However, this process is a time-consuming process. *In silico* tools developed under bioinformatics provide excellent estimation of the novel metabolites. antiSMASH is one of the powerful tools for the characterization of secondary metabolites in the bacterial genome (Blin *et al*., 2021; Medema *et al*., 2011). In this study, a comprehensive analysis of secondary metabolites in *Pseudomonas* sp. SXM-1 found in Xiamen Bay (China) was conducted. The results showed that *Pseudomonas* sp. SXM-1 genome contains not only siderophores, but also other biosynthetic gene clusters of novel secondary metabolites. The secondary metabolites revealed in this study may open a new avenue for fully understanding biosynthetic gene cluster functions in the bacterial genome.

In recent years, *Pseudomonas* spp. have been used in plant growth-enhancing studies. Goswami *et al*. (2013) used *Pseudomonas* spp. isolated from a marine ecosystem as a plant growth-promoting bacterium (PGPB). PGPBs can directly or indirectly affect plant growth. In the direct mechanism, there is the production of plant growth hormones and fixation of nitrogen by attaching to plant's roots, while the indirect mechanism is the production of metal chelating compounds such as siderophores. Because siderophores compete with pathogenic microorganisms for metal binding, they can neutralize these pathogens by chelating iron, which is essential for growth.

Pseudomonas spp. have also been used in agriculture as biocontrol agents against plant pathogens in agriculture. The study by Jin *et al.* (2013), the antagonistic property of *Pseudomonas* spp. against plant pathogens. *Pseudomonas* spp. was used as a biocontrol agent for root disease in Jerusalem artichoke. Jerusalem artichoke has low production cost and it is used in many fields, including production of alcoholic beverages. Therefore, the biocontrol of these plant pathogens is of great importance in agriculture and industry.

Riccardi *et al*. (2021) mentioned that *Pseudomonas* sp. TAE6080 inhibits biofilm formation of *Staphylococcus epidermidis*, which is a pathogen (Accession number of *Pseudomonas* sp. TAE6080 is JAHIDY010000002). *S. epidermidis* is a permanent member of the human microbiota, located on the skin and mucous membranes (Sabaté Brescó *et al*., 2017). They used the antiSMASH tool for the complete genome analysis and they found the gene clusters that have effects on the biofilm formation. Gene clusters related to biofilm formation were found as RiPP-like, NRPS, terpene, NAGGN, arylpolyene, redox-cofactor and betalactone. As a result of this study, it was observed that the formation of *S. epidermidis* RP62A biofilm decreased significantly. When compared with our study, it was seen that the gene regions of arylpolyene, RiPP-like, NRPS, betalactone, NRPS-like and redox-cofactor were common. Sabaté Brescó *et al.* (2017) used antiSMASH 5.0 version and their results were observed in 11 regions. However, when the same accession number was used with the antiSMASH 7.0.1 version, the results occurred in 14 regions.

Girard *et al.* (2023) investigated the phylogenetic trees and metabolic potentials of the *Pseudomonadaceae* family. They used antiSMASH 6.0 and ClusterBlast for the identification of biosynthetic gene clusters. In their study, gene clusters such as NRPS and RiPP-like were identified. In addition to those,

the presence of CLP (cyclopentadecanolide polymer) in the genomes of the strains *Pseudomonas* sp. SXM-1, *P. carnis* J380, and *P. aylmerense* B29B was revealed. Both Girard *et al.* (2023) and the present study utilized antiSMASH for analysis. However, while Girard *et al.* (2023) used antiSMASH 6.0, present study used antiSMASH 7.0.1. In the present study, the specific nucleotide regions of each gene and the associated genes have been analyzed in detail, and their potential effects have been discussed. Girard *et al.* (2023) demonstrated through phylogenetic and biosynthetic gene cluster (BGC) studies that most strains thought to belong to the *Pseudomonadaceae* genus are actually members of the *Halopseudomonas* or *Stutzerimonas* genera. Their work also identified 26 new species. While their study shares features such as MarR family transcriptional regulators, arylpolyenes, RiPPs, NAGGN, ectoine, NRPS, and PvdJ/PvdD/PvdP-like proteins with this study, advancements in antiSMASH versions allowed new gene clusters to be identified, including NRP-metallophores, redox-cofactor genes, and RiPP-like regions, which were not mentioned in the Girard *et al.* (2023) study.

In another study by Wu *et al*. (2016), the antibiofilm properties of *Pseudomonas* stutzeri 273 were studied. In this study, inhibiting the biofilm formation of *Pseudomonas aeruginosa* by another *Pseudomonas* genus, *P. stutzeri* 273 was studied. *P. aeruginosa* is a pathogenic bacterium that causes urinary tract infections, food poisoning and marine antifouling via biofilm formation. It has been found that exopolysaccharide EPS273 obtained from the marine bacterium *P. stutzeri* 273 prevents the formation of biofilm and disperses the formed biofilm.

Pan and Hu (2015) studied the new strain *Pseudomonas* sp. 10B238 which has the potential to produce antibiotics from deep-sea sediments, isolated from the South China Sea. They used the antiSMASH tool to determine antibiotics and secondary metabolites. A total of 11 potential sets of secondary metabolite biosynthetic genes have been predicted. NRP (siderophore), terpenes, arylpolyene, ectoine, bacteriocin were found.

Zeng *et al*. (2020) investigated the *Pseudomonas* sp. DMSP-1 genome. *Pseudomonas* species break down dimethylsulfoniopropionate (DMSP), the algal metabolite necessary to produce dimethyl sulfide (DMS). The results showed that while the genome contains 5510 protein-coding genes, enzyme-coding genes associated with DMSP catabolism were not found. Jain *et al*. (2023) studied the polyamine metabolizing rhizobacteria *Pseudomonas* sp. GBPI_506. 79 strains of the *Pseudomonas* sp. were compared with phylogenomic analysis. The common gene between *Pseudomonas* sp. strain GBPI_506 and *Pseudomonas* sp. SXM-1 is pyrroloquinoline quinone (PQQ) that is related to phosphorus availability, transport and also known as a redox cofactor (Wang *et al*., 2021). In this study, some parameters such as fresh shoot weight, leaf area, and root length were studied. It is clear that there is a difference between controlled plants and *Nicotiana benthamiana* threatened with *Pseudomonas* sp. strain GBPI_506. Currently, there is an increasing resistance to antibiotics because of the indiscriminate use of drugs. Fe *et al*. (2023), studied the effects of phase HZ2201 against *P. aeruginosa*, a clinically used gram-negative bacterium, were investigated. It was observed that HZ2201 has an inhibitor activity on the *P. aureginosa.*

Genome mining tools have been improving and they are widely used methods for *in silico* analysis. Microorganisms with known secondary metabolites are widely used in pharmaceutical and industrial studies. *Pseudomonas* spp. is one of these microorganisms and they are used in areas such as antifouling, agriculture and antibiotic production. Therefore, predicted secondary metabolites of *Pseudomonas* sp. SXM-1 were investigated in our study. Guo *et al*. (2021) also studied this species, but they focused on a single siderophore gene in the whole genome. In our study, all 14 regions of *Pseudomonas* sp. SXM-1 and almost all the secondary metabolites were examined. Thus, a much more comprehensive study was carried out compared with the study by Guo *et al* (2021). By examining all 14 regions, secondary metabolites that can be used in various fields were observed. Wet-lab characterizations of the secondary metabolites mentioned in this paper are strongly

recommended to microbiologists for confirmation.

AUTHORSHIP CONTRIBUTION STATEMENT

Levent Cavas: Conceptualization, Methodology, Validation, Writing - Original Draft, Writing-Review and Editing, Software, Visualization, Supervision, **Yağmur Bilgin:** Conceptualization, Methodology, Validation, Writing - Original Draft, Writing-Review and Editing, Software, Visualization, **İbrahim Kırkız:** Conceptualization, Methodology, Validation, Writing - Original Draft, Writing-Review and Editing, Software, Visualization,

CONFLICT OF INTERESTS

The authors declare that for this article they have no actual, potential or perceived conflict of interests.

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