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Species-Wide Genome Mining of *Pseudomonas putida* for Potential Secondary Metabolites and Drug-Like Natural Products Characterization

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Abstract

The gram negative bacteria species of *Pseudomonas putida (P. putida)* are important for heterologous expression of diverse biosynthetic pathways and numerous secondary metabolites biosynthesis. The genes code for such secondary metabolites biosynthetic proteins are organized in microbial genomes as clusters to bring the concerted expression of entire biosynthetic machinery. The complete and whole genome sequences of more than fifty different strains available in public DNA sequences databases provide an excellent opportunity to investigate the genetically encoded secondary metabolites potential of ecologically diverse *P. putida* strains. We implement the advance bioinformatics resources to annotate the so far available *P. putida* strains genomes for biosynthetic gene clusters (BGCs) and underlie secondary metabolites chemical scaffolds. The *P. puida* strains are found to harbor genomic signatures coding the molecular machinery for diverse secondary metabolites biosynthesis. The corresponding BGCs of these metabolites are found to be uniquely distributed across different *P. putida* strains speculate their role toward strain's ecological competency acquirement. The chemoinformatics dereplication and DrugBank database searching revealed the chemical mimicry of one putative metabolite with 2, 3, Dihydroxybenzoylserine, that mediates an antibiotic iron depletion along with human neutrophil lipocalin during innate immune response.

Keywords: Biosynthetic gene cluster; Secondary metabolites; *Pseudomonas putida*; Bacterial genomics

Introduction

Microbial secondary metabolites are small organic compounds play important role in the survival of microbial culture and ecological interaction with other organisms [1]. Microbial natural products are used as agricultural, dietary and pharmaceutical agents. These include nonribosomal peptides (NRPs), polyketides (PKs), post-translationally modified peptides (RiPPs), ribosomally synthesized compounds, saccharides, terpenoids and some hybrids natural products. The biosynthesis of these secondary metabolites are directed by group of genes physically located within a single locus on microbial chromosomal and plasmid DNA termed as Biosynthetic Gene Clusters (BGCs). These clusters allow the concerted expression of necessary biosynthetic, regulatory enzymes and transporter proteins require for biosynthesis, mechanism of action and transport of secondary metabolites [2]. The rich genetic diversity of microbial BGCs due to evolutionary basis eventually cause high chemical diversity in their underlie coding secondary metabolites [3,4].

The P. putida is a gram-negative bacterium typically colonizes at soil and aquatic habitats. The strains of this species exhibit wide metabolic versatility driving their adaptability to diverse habitats. The *P. putida* strains are important for the biosynthesis of variety of biotechnological significant natural products by heterologous expression of diverse biosynthetic pathways [5]. *P. putida* offer several particular advantages with respect to natural product biosynthesis, notably a versatile intrinsic metabolism with diverse enzymatic capacities and outstanding tolerance to xenobiotics. Therefore, *P. putida* strains are potential source of antimicrobial agents and implement in recombinant biosynthesis of valuable natural products [5,6].

The decreasing cost of genome-level DNA sequencing due to technological advancement in the form of next-generation sequencing allowed to understand the ecological diversity and dynamic functionality of microbial communities with increase resolution. Nowadays, these fast genome sequencing along with advance bioinformatics resources transformed the secondary metabolites discovery approaches towards more rational and predictive directions [7]. Moreover, the organization of BGCs as single locus on bacterial chromosomal and plasmid DNA actually eased these genomic motifs confident *in silico* predictions based on fundamental principles of bioinformatics [8]. However, still the genome-guided microbial secondary metabolites discovery and knowledge is limited compare to bioassay screening. In this study, we adopted multiple computational biology resources to mine all publically available whole genome sequences of *P. putida* strains to elucidate the secondary metabolites coding potential of this species. The chemical scaffolds of putative secondary metabolites as identified are further evaluated for drug-like potential using chemoinformatics resources.

Material and Methods

Genome sequences retrieval

The complete and partial genomes assemblies of 58 different stains and isolates of *P. putida* are retrieved from NCBI Genbank [9] and Integrated Microbial Genome (IMG) databases [10]. The data retrieval was made in March 2016.

Secondary metabolites prediction

Several prediction models are implemented in bioinformatics resources for putative identification of BGCs responsible for

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secondary metabolites biosynthesis. AntiSMASH 3.0 is a robust and comprehensive bioinformatics resource for identification and annotation of known and putative unknown BGCs using probabilistic algorithm [11]. Likewise, BAGEL3 and PRISM are specifically designed for bacteriocin and type II polyketides putative cluster identification respectively [12,13]. Complementation of AntiSMASH with these specialized prediction resources provides confident prediction regarding microbial secondary metabolites coding potential [14]. These platforms implement different databases, like working on the basis of same principles of hidden Markov model (HMM) [15], BLAST algorithm [16], PFAM [17], GenBank [18] and UniprotKB [19] for BGCs annotation from genome sequences.

The AntiSMASH constructs a database of currently known BGCs throughout the tree of life from the 'Minimum Information about a Biosynthetic Gene cluster' (MIBiG) community project (http://mibig.secondarymetabolites.org). The latest AntiSMASH version integrated with ClusterFinder and ClusterBlast modules is capable now to identify putative unknown types of BGCs with similarities to known BGCs and end chemical product detail [11].

The PRISM resource implement greedy algorithm to assemble the metabolites biosynthetic domains into BGCs. The putative BGCs are detected when all the necessary domains of chain length factor, ketosynthase (KS), condensation and substrate-activating adenylation, acyl-adenylating, acyltransferase domains are fill full. PRISM annotate the bacterial genomes for BGCs by analyzing open reading frames with a library of hundreds of hidden Markov models and curated BLAST databases. Later for each list of open reading frames a set of combinatorial plans are generating. These combinatorial scaffold libraries are cheminformatically dereplicated to a database of known natural products, while the clusters are dereplicated against a database of known BGCs using a multilocus sequence typing-style algorithm [13]. The PRISM is especially designed for the prediction of polyketide and nonribosomal peptides types of metabolites.

The BAGEL3 resource is useful for prediction of bacteriocin type of secondary metabolites [12]. The tool utilizes three different databases containing modified, unmodified bacteriocins and post-translationally modified peptides (non-bactericidal). The current version of BAGEL3 implements both direct and indirect genome mining approaches to perform high quality BGCs annotation. The indirect approach implements simple ORF while the direct approach utilizes Glimmer ORF call. Later BLAST search [16] is performing against the abovementioned three databases and the context is finally annotated using PFAM database [12].

Chemical structures elucidation

The chemical structures of the putative secondary metabolites as identified from above-mentioned resources are elucidated using ChemDraw chemoinformatics tool [20]. The predicted chemical structures are converted to SMILES format for downstream analyses.

DrugBank database screening

The putative secondary metabolites are evaluated for their respective drug targets and homologue drug-like chemical scaffolds identification using DrugBank resource [21].

Molecular docking and drug-like potential evaluation

The MOE (Molecular Operating Environment; Chemical Computing Group Inc. Canada, 2016) (Version 2014.09) resource used to infer the drug like potential of putative secondary metabolites based on essential *Lipinski's rule of five* [22] and to understand the binding mode with respective drug target by calculating the molecular docking scores, binding energies and binding affinities.

Results and Discussion

P. putida species genomes

During the time of this analysis total 58 different strains of *P. putida* genomes were available in NCBI and IMG databases. Sixteen of these were complete genome, while the rest were partial or whole genome sequence assemblies. In addition, 14 plasmids sequences of *P. putida* isolates were also retrieved (Table S1).

Secondary metabolites analyses

The *P. putida* strains genomes data scan revealed the BGCs coding for nonribosomal peptide synthetases (NRPs), polyketide synthase (PKs), bacteriocin, homoserine lactone (HSL), phenazine, arylpolyene, terpene, ectoine, and some other types of secondary metabolites (Table S2). Moreover several hybrid BGCs i.e., originated by fusion of sub-clusters coding for distinct classes of secondary metabolites, include hybrid butyrolactone-HSL, hybrid phenazine-Nrps, hybrid bacteriocins-NRPS and BGCs of unknown biosynthetic capabilities that need additional functional elucidation were also identified.

Putative nonribosomal peptides, polyketides and bacteriocins types of BGCs: The nonribosomal peptides (NRPs) are diverse class of secondary metabolites reported as toxins, drugs, siderophores and pigmentation agents. NRPs are synthesized on nonribosomal peptide synthetase (NRPS) complex, which is a multidomain enzyme [23]. The *P. putida* strains utilized for the production of complex and industrially important NRPs to get advantage from their hetrologous expression and non-pathogenic nature and to avoid any inconvenience by working with pathogenic strains [24]. The analyses of publically available *P. putida* strain's genomes sequences revealed their ability to synthesize diverse set of NRPs biosynthesis. The PRISM and antiSMASH resources identified 49 and 50 NRPs coding BGCs respectively for the genomes dataset archives of *P. putida* strains (Table S2 to S4).

Another large multi-domain enzyme complex responsible for bioactive polyketides natural products biosynthesis is known as polyketide synthases (PKS). Polyketides are considered as important metabolites in human medicine and been widely used to treat acute and degenerative diseases. Three types of PKS are know so far in bacteria species. The type I PKS are organized into modules catalyzes non-iteratively the reduced polyketide chain elongation and synthesis, such as erythromycin-A. Type II PKS carry a single set of iteratively acting aromatic polyketides biosynthetic domains, exemplified by tetracenomycin-C. The type I and II PKS employ acyl carrier protein (ACP) to activate acyl CoA substrates for the growth of polyketide intermediates. The type III PKS also hold iteratively acting enzymes responsible for aromatic polyketides biosynthesis independent of ACP [25]. The *P. putida* is reported as suitable host for the heterologous production of polyketides type of metabolites [5]. The antiSMASH and PRISM analyses for *P. putida* genome dataset revealed total 5 and 22 type 1 PKS BGCs respectively (Table S2 and S4). Though PRISM is especially designed resource for type II polyketides prediction, but none of the *P. putida* strain genome annotated for type II or III polyketides BGCs.

Bacteriocins are low-molecular weight antimicrobial ribosomally synthesized peptides produced by bacteria and archaea species. Bacteriocins are mainly use as food preservative and antibiotics

[26,27]. Bacteriocins are diverse in chemical structures and mode of action and divided into four sub-groups. Class-I bacteriocins are post-translationally modified peptides been reported with antimicrobial activity such as sactipeptides, lanthipeptide and lasso peptides [28]. Class-II bacteriocins are antimicrobial peptides without post-translational modification and subdivided into four sub-classes: subclass IIa, the pediocin-like bacteriocins; subclass IIb, two component antimicrobial peptides; subclass IIc, circular bacteriocins and subclass IId, linear non-pediocin-like single-peptide antimicrobial peptides [29]. The class-III bacteriocins are heat-labile proteins with the size of >10 kDa, also called as bacteriolysins [30]. These bacteriocins show homology to endopeptidases and specificity with the target cells at the C-terminal domain proteins [31]. Class-IV bacteriocins are cyclic bacteriocins with post-translational modification [32]. We observed total 25 bacteriocins coding clusters for all the complete and partial genomes dataset of *P. putida* strains from BAGEL3 resource analysis (Table S3). Most of these are annotated as class-III bacteriocins with molecular weight>10 kD showing similarity with zoocin-A, colicin and putidacin-L1. Some *P. putida* strains, like ABAC8 and PC2 are shown to produce class-II bacteriocins exhibit similarity hit with propionicin-SM1. The AntiSMASH identified total 80 bacteriocins coding clusters for the collected *P. putida* genomes dataset (Table S2). In addition to simple bacteriocins, two hybrid clusters of bacteriocins were identified for *P. putida* strains MC4-5222 and PC2. A lantipeptide-bacteriocin hybrid cluster was annotated for genome assembly of *P. putida* MC4-5222. The lanthipeptides are ribosomally synthesized and post-translationally modified peptide (RiPP). These are classified as class-I bacteriocins with antibacterial activities [33]. The lantipeptide-bacteriocin produces a hybrid natural product holding conceivable potential to be used as therapeutics [34]. Another hybrid bacteriocin, i.e. bacteriocin-Nrps cluster is predicted for *P. putida* strain PC2. This cluster shown 83% similarity with WLIP (White Line Inducing Principle) BGC (MIBiG ID: BGC0000462). The WLIP BGC are reported to attribute the antagonism function to microbe against xanthomonas and involve in swarming and biofilm formation in the *Pseudomonas* species. The bacterial strains holding molecular signatures for WLIP are reported to exhibit antifungal activity [35].

***P. putida* strain-specific BGCs:** A distinct pattern of BGCs existence was observed among *P. putida* strains. For instance only four acyl homoserine lactone (AHL) clusters identified for genome assemblies of three strains, i.e. one each for *P. putida* PD1 and UASWS0946, while two clusters for *P. putida* FDAARGOS_121 sequences. Likewise, a hybrid butyrolactone-hserlactone hybrid BGC detected in complete genome assembly of *P. putida* PC2. The homoserine lactones produced by many gram-negative bacteria species play central role in the quorum sensing mechanisms that relay on community based signaling for development of symbiotic as well as pathogenic interaction with eukaryotic cell [36]. It has been reported that majority of the *P. putida* lack LuxR and its cognate LuxI homolog modular proteins and not equipped with AHL quorum sensing system except some plant growth promoting beneficial strains, i.e. *P. putida* WCS358 [37,38]. Here the genome sequence analyses of 58 different strains/isolates for AHL producing BGCs also revealed limited number of *P. putida* capable to rely on AHL based quorum sensing. The *P. putida* PD1 strain is reported as beneficial endophytic involve in promoting plant growth and protection against the phytotoxic effects of phenanthrene [39]. We speculate that *P. putida* PD1 might have acquired such physiological capabilities from quorum sensing mechanisms based on the AHL cluster as predicted from our analysis. Likewise, the *P. putida* FDAARGOS_121 is isolated from infectious habitat of female urinary track as according to NCBI bioproject detail of this isolate. Several opportunistic pathogenic clinical isolates of *P. putida* reported to produce dense biofilms to acquire antibiotic resistance [40]. Moreover, a study suggested that AHL depended quorum-Sensing system greatly influence the biofilm structural development in several clinical *P. putida* isolates [41]. Therefore, based on this scenario, we assume that the two homoserine lactone clusters as identified for genome assembly of *P. putida* FDAARGOS_121 strain might play a role in biofilm formation of this clinical isolate to combat the effect of antibiotics.

Another strain-specific distinction observed for phenazine coding BGCs and one each of such BGC annotated for genome assemblies of *P. putida* strains, i.e. W619, KG-4, SQ1 and FDAARGOS_121. In addition, a hybrid phenazine-Nrps BGC annotated for genome assembly of *P. putida* FDAARGOS_121 showing 100% sequence similarity with pyochelin BGC (MIBiG ID: BGC0000412). Pyochelin (PCH) is a siderophore synthesized by *P. aeruginosa* to develop high affinity for iron scavenging [42]. Phenazines secondary metabolites produce by variety of bacteria, especially pseudomonads, which have diverse applications, such as electron acceptors and donors and environmental biosensors. In addition, the phenazines have broad spectrum antibiotic properties and play role in virulence induction [43].

A single cluster each for terpene and ectoine type of secondary metabolites detected for genome assemblies of *P. putida* S610 and UASWS0946 strains respectively. The terpene biosynthetic cluster of *P. putida* S610 is showing 85% sequence similarity with carotenoid BGC (MIBiG ID: BGC0000642) as revealed by clusterBlast module of AntiSMASH. Terpenes are plant or fungal metabolites and small number of odoriferous terpenes of bacterial origin are identified so far. The bacterial terpenes are antimicrobial in nature and used in fermentation industries [44]. Mostly the bacterial species are not producing the detectable amount of terpenes, however metabolically engineered *P. putida* used as a host for the production of monoterpenoids [45]. We found no comprehensive report about terpeniods coding BGCs annotation in genomic sequences of *P. putida* S610 strain. Likewise no report is available about terpenes purification and biosynthesis from this strain.

The ectoine (natural cell protectant) is a compatible solute acts as an osmolyte that capable the bacterium species to survive in extreme saline environmental stress [46]. The ectoine biosynthetic genes are almost found exclusively in bacteria species. In a pilot study about the whole-genome information of *P. putida* strain UASWS0946, though several genes for antimicrobial biosynthesis are addressed but genomic loci associated with the ectoines biosynthesis are not discussed [47]. The analysis inferred that the ectoine biosynthetic cluster of *P. putida* UASWS0946 showing less than 20% similarity with other *Pseudomonas* species and assume strain specificity. Likewise, the cluster is not showing similarity with MIBiG repository and found to be comprised of several hypothetical proteins coding genes. Though current prediction not fully validate the ectoines biosynthesis of *P. putida* UASWS0946, but the existence of corresponding molecular signature of this metabolite somehow speculate about *P. putida* UASWS0946 or its ancestral strains ectoines biosynthetic capabilities.

The xantholysin BGC (i.e. MIBiG ID: BGC0000463) detected for genome assemblies of *P. putida* 1A00316 and BW11M1 strains. Xantholysins are structurally diverse cyclic lipopeptide congeners produced by several *Pseudomonas* strains to exhibit biocontrol effect on phytopathogenic fungi [48]. A mixture of these secondary metabolites have experimentally purified from *P. putida* BW11M1 strain and reported [49]. However, no in-depth analysis regarding xantholysins

coding BGCs of *P. putida* BW11M1 strain performed in the recently published complete genome sequence information of this isolate [50]. The xantholysins coding BGC found to exhibit sequence similarity with *P. mosselii* SJ10 strain as inferred from clusterBlast module of antiSMASH. This depicts somehow about the specialized xantholysin biosynthesis capability of *P. putida* 1A00316 and BW11M1 strains only.

The pseudopyronine-A coding cluster (i.e. MIBiG ID: BGC0001285) detected for genome assemblies of *P. putida* 1A00316 and BW11M1 strains. According to the clusterBlast module of AntiSMASH, this cluster as a whole showing less than 20% sequence similarity with other Pseudomonas species and revealed the distinction of *P. putida* 1A00316 and BW11M1 strains in term of pseudopyronine-A biosynthetic potential compare to rest of *P. putida* strains. The pseudopyronines A and B types of antibiotics previously isolated from the plant-derived *P. putida* BW11M1 strain. These pseudopyronines are 3, 6-dialkyl-4-hydroxy-2-pyrones and showing antimicrobial activities against numerous human and some plant pathogens, i.e. *P. savastanoi* [51]. The molecular signatures existence of such active secondary metabolites in *P. putida* 1A00316 and BW11M1 make these strains venerable to experimentally examine their pseudopyronine biosynthesis capabilities.

The entolysin BGC (MIBiG ID: BGC0000344) detected for genome assemblies of *P. putida* 1A00316 and BW11M1 strains. This BGC also showed no sequence similarity hit with other *P. putida* and inferred strain specificity. Entolysin is a cyclic lipopeptide, reported to produce by entomopathogenic *P. entomophila*. The strong hemolytic activity of the *P. entomophila* is linked to its entolysin production. The genome sequence analysis suggested the close relationship of *P. entomophila* with *P. putida* [52]. Moreover, the *P. putida*1A00316 is reported to be quite distinct in virulence and metabolism compare to other *P. putida* strains and exhibit nematicidal activity *[53]. Though* diverse nematicidal factors are addressed in genome sequence report of *P. putida* 1A00316 isolate [53], but the entolysin biosynthetic molecular signatures and capabilities of this isolate not discussed. W*e assume the role of predicted* entolysin coding BGC in nematicidal activity of *P. putida*1A00316 strain.

The orfamide biosynthetic cluster (i.e. MIBiG ID: BGC0000399) detected for genome assembly of *P. putida* S610 and BW11M1 strains only. The orfamides are cyclic lipopeptides (CLPs) biosurfactants cause oomycete zoospores lysis and act as biocontrol agent and insecticidal [54]. The *P. putida* S610 and BW11M1 strains are plant-borne and assume to harbor an insecticidal orfamide biosynthetic cluster to participate in host plant defense and growth mechanisms.

A delftibactin coding cluster detected in case of genome sequence analysis of *P. putida* B001 found to show considerable sequence similarity (i.e. 42%) with standard MIBiG repository (i.e. cluster ID: BGC0000984). The delftibactin coding cluster exhibits no significant sequence similarity with other *Pseudomonas* species and seems to be a specific genomic feature of *P. putida* B001. The *P. putida* B001 isolate is reported as rhizobacterium isolated from yellow sea shore somewhere in Korea and reported to induce plant defense against bacterial, fungal and viral diseases [55]. The delftibactin metabolite promotes bacterium cellular defense and protection against toxic effects of gold and cause gold biomineralization [56,57]. The strain's ecology and biological attribution reveal up to some extent that *P. putida* B001 might develop or acquired a genetic toolkit in the form of delftibactin coding BGC to regulate gold metal homeostasis and reduce gold toxicity. However such abilities of the isolate has not been discussed or tested so far.

Common pyoverdine coding BGC in *P. putida* strains: A cluster with MIBiG ID, i.e., BGC0000413 code for pyoverdine type of secondary metabolite and observed in all types of *P. putida* strains (Table S2). Pyoverdine is water-soluble, fluorescent pigment of the fluorescent Pseudomonas species. It is a powerful iron (III) scavenger and an efficient iron (III) transporter. As a fluorescent pigment, it acts as a powerful marker for bacterial differentiation as a siderophore. The existence of pyoverdine coding BGCs in all *P. putida* strains reveal its basic important in satisfying the absolute iron requirement of these strictly aerobic bacterial species [58,59].

Plasmids DNA screening for secondary metabolites coding gene clusters: The horizontal gene transfer (HGT) phenomenon shaping the secondary metabolites coding gene clusters distribution particularly in fungal species [60]. Likewise the pseudomonads generally reported for their ability to exhibit diverse metabolic capacities by exchange of genetic information through plasmid based HGT [61]. This speculates the existence of BGCs molecular signatures on *P. putida* strains plasmid DNA molecules. Therefore, we screened the available *P. putida* plasmid replicon sequences for potential BGCs annotation. However, none of the prediction model clued towards an existence of any BGC motif for available plasmid sequences of *P. putida* strains. This revealed limited capabilities of *P. putida* BGCs horizontal gene transfer (HGT) via plasmids replicons.

Drug-like potentials of putative secondary metabolites

Besides high molecular weight metabolites, two drug-like compounds are characterized for genome datasets of certain *P. putida* strains. The Compound-1 (Figure 1A) predicted for genome assembly of *P. putida* S610, while Compound-2 (Figure 1B) for genome assemblies of *P. putida* 1A00316 and BW11M1 strains. These compounds encode from NRPs types of BGCs. The drug-like properties of these metabolites are established as according to essential Lipinski rules of five [22]. The molecular weight of these metabolites are less than 500 daltons and the number of hydrogen bond donors and acceptors are less than 5 and 10 respectively. Likewise the value of log *P* (octanol-water partition coefficient) is not greater than 5. Furthermore detail of these properties given in (Table 1). The Compound-2 coding BGC found to show 100% sequence similarity with *P. mosselii* SJ10 strain. The *P. mosselii* SJ10 is a caprolactam-degrading species and the complete genome sequence of this bacterium though provide the basis of aromatic compound biodegradation mechanisms [62], but no information about such drug-like natural products biosynthesis been addressed.

To further investigate the drug-like potential of these metabolites, the DrugBank database was searched [15]. The DrugBank database scanning revealed that Compound-1 hold structural similarity with 2,3,-Dihydroxybenzoylserine (DHBS), which is a catecholate-type of siderophore synthesize by many microbes to obtain iron from the environment [63]. It is been reported that human neutrophil gelatinase-associated lipocalin (NGAL) bind DHBS and the NGAL:Fe-DHBS complex act as a potent antibacterial agent by iron sequestration and conduct an iron-depletion strategy during innate immune response [64].

Putative Compound-1	Putative Compound-2
Weight: 322.32 log P: -0.62 log S: -1. 26 Hydrogen Donor: 4 Hydrogen Acceptor: 6 Toxic: No	Weight: 310.306 log P: -0.68 log S: -1. 69 Hydrogen Donor: 4 Hydrogen Acceptor: 6 Toxic: No

Table 1: The drug-like chemical characteristics of putative Compound-1 and Compound-2.

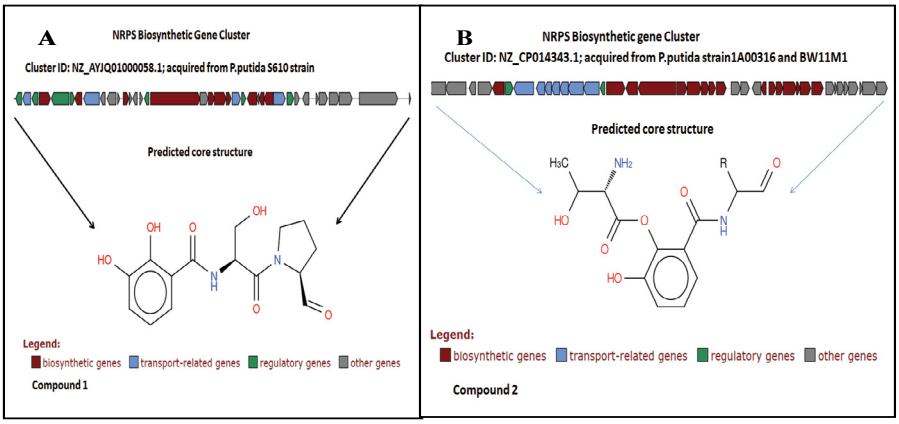

Figure 1: (A) Drug like Compound-1 [2, 3-dihydroxy-N-((S)-3-hydroxy-1-((S)-2-(hydroxymethyl) pyrrolidin-1-yl)-1-oxopropan-2-yl) benzamide] and **(B)** Compound-2 [(2S)-2-hydroxy-6-((1-oxopropan-2-yl) carbamoyl) phenyl 2-amino-3-hydroxybutanoate] detected for *P. putida* S610 and *P. putida* 1A00316, BW11M1strains genome assemblies respectively.

Legend	Docking score (S)	Binding affinity Kcal/mol	Binding energy Kcal/mol	Molecular weight g/mol	Hydrogen Donor	Hydrogen Acceptor
DHBS	-12.5818	-3.45	-18.90	240.19	4	6
Compound-1	-10.6557	-3.98	-16.50	322.32	4	6

Table 2: Molecular docking scores (S), binding energies Kcal/mol, binding affinities Kcal/mol and drug like properties comparison for putative compound-1 and 2, 3-dihydroxybenzoylserine.

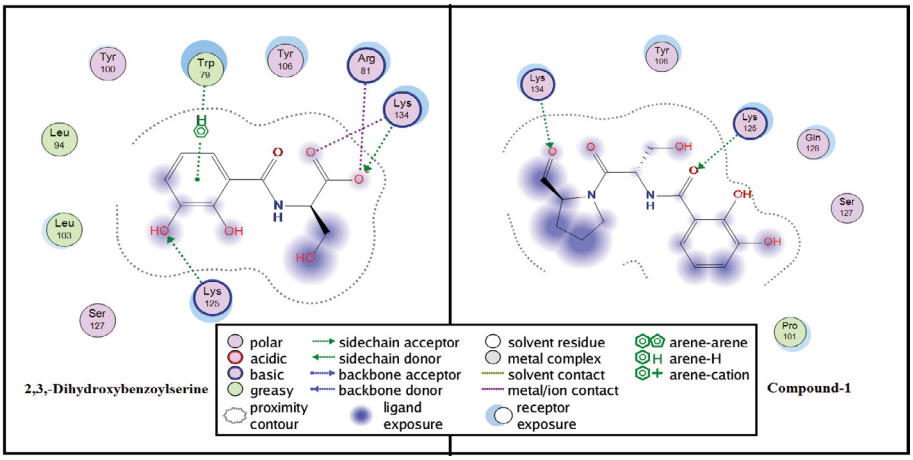

Figure 2: The molecular docking poses of DHBS (left) and putative compound-1(right) within NGAL binding pockets.

To further infer the receptor binding potential of putative Compound-1 in-comparison to DHBS, we implement the molecular docking analysis. The complex structure of the NGAL:Fe-DHBS retrieved from PDB (Protein Data Bank) [ID: 1L6M] for this purpose. The docked poses of Compound-1 exhibit favorable interaction with critical residues (i.e. Lys^{125} and Lys^{134}) like DHBS in NGAL catecholate-type siderophore binding pocket. The docking scores, binding energy and binding affinity of putative Compound-1 and DHBS are found relatively similar and showed no significant difference (Figure 2 and Table 2). This speculate that *P. putida* strain S610 synthesize the putative Compound-1 as catecholate-type siderophore to sequester iron from the environment.

Conclusion

Currently, the genome-sequences availability of diverse strains of the *P. putida* species provides an excellent opportunity for comprehensive comparisons of their biosynthetic potential. The genome-wide distribution of BGCs revealed that *P. putida* strains harbor operons for coding diverse set of secondary metabolites. These candidate secondary metabolites coding clusters provide valuable targets for experimental examination to exploit new resources. Additional methodologies are required to decipher these biosynthetic genome motifs into corresponding compounds to open a new era in the discovery of secondary metabolism.

Competing Interests

The authors have declared that no competing interests exist.

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