Draft genomes and initial characterization of siderophore producing pseudomonads isolated from mine dump and mine drainage

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Siderophores are of high interest for biotechnological, pharmaceutical, agricultural and industrial applications. Although they are synthesized by various organisms, the yield is usually low which hinders their suitability for broad range uses. Thus, it is necessary to identify novel producers and to increase the understanding of the biosynthesis pathways. Herein we report the isolation of two novel \textit{Pseudomonas} strains and the identification of the gene clusters for the biosynthesis of pseudomonine as well as pyochelin and pyoverdine.

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Iron is an essential element for living organisms and involved in numerous cellular processes. Thus, the maintenance of a proper intercellular level is critical [1]. However, iron is not easily bioavailable due to the formation of insoluble forms at aerobic conditions and neutral pH, although it is one of the most abundant elements. One strategy to circumvent this issue is the employment of small carriers called siderophores [2]. These are produced as secondary metabolites by various organisms including prokaryotes (bacteria [3,4], cyanobacteria [5] and archaea [6]) as well as eukaryotes (plants [7], fungi [8] and mammals [9]). Siderophores differ in structure and chemical properties but can be classified according to their metal chelating functional groups into hydroxamate, catecholate, carboxylate and mixed type [10]. The chemical structure also defines their affinity for iron but also other metals. Besides their physiological role, they have been shown to be promising candidates for various industrial, agricultural and medical applications [4,11]. However, the biosynthesis pathways of siderophores are usually complex and the yield is modest. Thus, it is currently laborious and expensive to produce siderophores in sufficient amounts. Therefore, the isolation, identification and characterization of siderophore producers is of high importance to facilitate an application of these compounds. Especially pseudomonads are known to be versatile siderophore producers [12]. In this study, we isolated two novel strains that offer a steady siderophore production rate and especially showed a good affinity for other metals like Al and Cu.

The herein described strains have been isolated from a heap in “Neuhausersdorf” (strain H3, 50°55’07.1”N 13°22’19.2”E, 2016/12/13) and wet soil next to the mine drainage region “Roter Graben” in Tuttendorf (strain RGB, 50°56’24.1”N 13°22’19.6”E, 2017/01/18). They were selected as potent siderophore producers by evaluation on Chrome azurol S (CAS)-agar plates [13,14] (Fig. 1). Further, they showed to be selective for other metals than iron, making them interesting candidates for further investigations.

Genome sequencing was done to reveal the genus and to gain information about siderophores types that are supposed to be produced. Therefore, both strains were cultivated in 50 ml LB media, harvested after 3 days and DNA was isolated as described previously [15].

The DNA library preparation, genome sequencing, assembly, annotation and analysis were done as described previously [16]. Therewith a genome coverage of 247x for strain H3 and 249x for strain RGB was obtained, respectively. The results of the genome sequencing are summarized in Table 1. For isolate H3, 49 contigs (49 > = 1000 bp) were identified that cover about 5.8 Mbp with an average G + C content of 58.8 % (N50 = 175970; N75 = 108569;
Both strains isolated by Hofmann et al. [39] L50 = 11; L75 = 21. For isolate RGB, the assembly resulted in 43 contigs (41 > 1000 bp) covering about 6.3 Mbp with an average G+C content of 60.5 % (N50 = 359427; N75 = 161348; L50 = 6; L75 = 12). A 16S rDNA-based phylogenetic analysis was done for both isolates revealing that both are classified as Pseudomonas species (Fig. 2). Isolate H3 clusters together with P. rhodesiae (99.3 % identity to strain DSM 14020) and P. grimonii (99.2 % identity to strain DSM 17515). However, strain H3 forms a separate branch and might be related to a novel species, which has to be proven. Isolate RGB is situated close to P. auricularis NBRC 10220 (100 % identity to strain NBRC 10220) and is therefore probably associated to this species.

Biochemical typing of both strains was done by Api20 NE (Table 2). Most of the metabolic properties of the isolates resemble those of the reference strains. Both strains are Gram-negative as well as catalase and oxidase positive. Notable differences can be found for isolate H3 in comparison to the nearest relatives on 16S rDNA base. It does not show growth on L-Arabinose and N-acetylglucosamine, while species rhodesiae and grimonii are able to use these compounds as carbon source. Further, strain H3 did not show fluorescence on Kings B or CSGA medium [17] what indicates that this strain is producing a non-fluorescent siderophore. Strain RGB showed fluorescence only on CSGA media which indicates that this strain might produce fluorescent siderophores. Both strains are able to grow on beech wood hydrolysate (BWH) as cheap alternative to glucose as carbon source for the production of siderophores [18] (Fig. 3). Therefore, both strains were cultivated on minimal media [19] containing 5 mM BWH for 25 h at 30 °C and 160 rpm. The doubling time is with 1.75–2.25 h in a comparable order of magnitude to glucose-grown pseudomonads (Table 3).

**Table 1** Genome statistics of the isolated pseudomonads.

| Genome Feature          | Pseudomonas sp. H3 | Pseudomonas sp. RGB |
|-------------------------|---------------------|---------------------|
| Sum of contig length (bp) | 5,865,301           | 6,336,192           |
| Contigs                  | 49 (> 3000 bp)      | 43 (> 950 bp)       |
| G + C content (%)        | 58.8                | 60.5                |
| Protein coding genes     | 5361                | 5723                |
| Average gene length (bp) | 950                 | 960                 |
| Coding percentage (%)    | 87                  | 87                  |
| tRNAs                   | 42                  | 44                  |
| rRNAs                   | 3                   | 3                   |

**Fig. 1.** Isolates H3 (left) and RGB (right) plated on CAS-agar selection plates. Production and secretion of siderophores by the bacteria is indicated by formation of yellowish halos around the colonies.

**Fig. 2.** Separated subtrees with focus on related strains of isolate H3 (A) and isolate RGB (B). Phylogenetic analysis of the isolates H3 and RGB was done based on a 16S rDNA sequences. The complete multiple sequence alignment was prepared with 167 16S rDNAs by applying the ClustalW algorithm. The maximum likelihood tree was constructed by using the MEGAX software and bootstraps of 5,000 replicates [39]. Bootstrap values above 50 % are indicated.
Table 2
Growth of Pseudomonas isolates in comparison to Pseudomonas type strains.

| Pseudomonas sp. H3 | Pseudomonas sp. 50090 | P. fluorescens DSM 17717 | P. marginalis DSM 13124 | P. poae DSM 14396 | P. simiae DSM 18861 | P. rhodesiae DSM 14020 | P. grimontii DSM 17515* |
|------------------|----------------------|-------------------------|------------------------|-------------------|----------------------|-----------------------|------------------------|
| Growth at 4 °C   | +                    | +                       | +                      | +                 | +                    | +                     | +                      |
| Growth at 20 °C  | +                    | +                       | +                      | +                 | +                    | +                     | n.d.                   |
| Growth at 37 °C  | –                    | –                       | –                      | –                 | –                    | –                     | n.d.                   |
| Fluorescence on Kings B media | –   | –                       | +                      | –                 | +                    | +                     | n.d.                   |
| Fluorescence on CSGA media | –   | +                       | n.d.                   | n.d.              | n.d.                 | n.d.                  | n.d.                   |
| Oxidase test     | +                    | +                       | –                      | +                 | +                    | +                     | +                      |
| D-Glucose        | +                    | +                       | –                      | +                 | +                    | +                     | +                      |
| L-Arabinitol     | –                    | +                       | –                      | +                 | +                    | +                     | +                      |
| D-Mannose        | +                    | +                       | +                      | +                 | +                    | +                     | +                      |
| Rhamnose         | –                    | –                       | –                      | –                 | n.d.                 | –                     | –                      |
| Trehalose        | +                    | –                       | +                      | –                 | +                    | –                     | +                      |
| Succinate        | +                    | –                       | +                      | –                 | +                    | –                     | +                      |
| D-Mannitol       | +                    | +                       | +                      | –                 | +                    | +                     | +                      |
| N-Acetilglycosamine | –          | –                       | –                      | +                 | –                    | +                     | +                      |
| D-Maltose        | –                    | –                       | –                      | –                 | –                    | –                     | –                      |
| Potassium gluconate | +                  | +                       | +                      | +                 | +                    | +                     | +                      |
| Decanoic acid    | +                    | +                       | +                      | +                 | +                    | +                     | n.d.                   |
| Adipic acid      | –                    | –                       | –                      | +                 | –                    | –                     | –                      |
| Malic acid       | +                    | +                       | –                      | –                 | +                    | +                     | n.d.                   |
| Trisodium citrate| +                    | +                       | +                      | –                 | +                    | +                     | +                      |
| Phenylacetate    | –                    | –                       | –                      | –                 | –                    | –                     | –                      |
| Benzoate         | +                    | –                       | +                      | –                 | n.d.                 | n.d.                  | n.d.                   |
| Serine           | +                    | +                       | +                      | +                 | n.d.                 | n.d.                  | n.d.                   |
| Decane           | +                    | –                       | +                      | n.d.              | n.d.                 | n.d.                  | n.d.                   |

(+) growth; (–) no growth; (+–) slow growth; (n.d.) not determined; *determined for at least 90 % of the isolated strains.

Fig. 3. Growth of (A) Pseudomonas sp. H3 and (B) Pseudomonas sp. RGB on 5 mM beech wood hydrolysate at 30 °C and 160 rpm.

Table 3
Doubling times of Pseudomonas isolates and other Pseudomonas strains.

| Strain                  | Doubling time in h | Substrate                  | References          |
|------------------------|--------------------|----------------------------|---------------------|
| Pseudomonas sp. H3     | 2.25               | Beech wood hydrolysate     | This study          |
| Pseudomonas sp. RGB    | 1.75               | Beech wood hydrolysate     | This study          |
| P. fluorescens        | 1.42               | Glucose                    | [20]                |
| P. putida              | 1.67               | Glucose                    | [21]                |
| P. fragi               | 0.83               | Glucose                    | [22]                |
| P. chlororaphis       | 1.25               | Glucose                    | [23]                |

The biological subsystem distribution of the annotated genes based on RAST can be found in Table 4 [24]. A subsystem coverage of about 50 % was achieved for both isolates. Herein, 1.5 % of the genes of strain H3 and 2.4 % of the genes of strain RGB are supposed to be related to iron acquisition and metabolism, respectively. An additional genome analysis was executed on the antismash 5.0 platform to estimate the production of secondary metabolites and especially siderophores [25]. For Pseudomonas sp. H3, twelve
secondary metabolite gene clusters were identified whereas four are annotated as putative siderophore gene clusters (cluster 1, 4, 9 and 10; Table 5). Cluster 1 contains several genes that can be found in the pyoverdine biosynthesis cluster, but no relevant peptide synthetase. Thus, it is unlikely that pyoverdines can be produced, especially as strain H3 does not show fluorescence. Cluster 2 and 9 contain lcuA/lucC-like synthetases, which indicate the production of hydroxamate siderophores like aerobactin and desferrioxamine. This remains to be proven as these clusters also do not contain related biosynthesis components like an N-hydroxylase or a decarboxylase. At least, manual annotation showed that only cluster 10 contains the gene set for the production of the isoxazolidone siderophore pseudomonine (Table 6) [26–29]. This siderophore was found in Pseudomonas entomophila and Pseudomonas fluorescens strains [30]. However, not much is known about the biochemical and metal binding properties of this siderophore.

For strain RGB, twelve secondary metabolite gene clusters were identified whereas three are annotated as putative siderophore gene cluster for the production siderophores (cluster 1, 2 and 8; Table 7). A manual analysis on genome level allowed for the identification of siderophore gene clusters contain the relevant genes for production of pyochelin (cluster 2) and pyoverdine (cluster 1 and 8). The annotation of the respective genes can be found in Tables 8 and 9. The pyochelin cluster is complete [31,32]. However, the cluster organization of pyoverdine is different in strain RGB compared to the reference in Pseudomonas aeruginosa PA01. Some regulatory genes and the cluster pvcABCD (relevant for the biosynthesis of the pyoverdine chromophore) are missing [33]. The fragmentation of the clusters might be a result of the draft genome sequence. However, all required genes that are needed for the biosynthesis and transport of pyoverdine are present in strain RGB [34]. Pyochelin as well as pyoverdines are known to be

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**Table 4** Biological subsystem distribution of annotated genes in the isolates H3 and RGB.

| Code | Subsystem coverage | Pseudomonas sp. H3 | Pseudomonas sp. RGB |
|------|--------------------|--------------------|--------------------|
|      | Description        | Value | Percent | Value | Percent |
| A    | Cofactors, vitamins, prosthetic groups, pigments | 317 | 7.8 | 348 | 8.1 |
| B    | Cell wall and capsule | 214 | 5.2 | 180 | 4.2 |
| C    | Virulence, disease and defense | 166 | 4.1 | 153 | 3.5 |
| D    | Potassium metabolism | 30 | 0.7 | 27 | 0.6 |
| E    | Miscellaneous | 100 | 2.5 | 84 | 1.9 |
| F    | Phages, prophages, transposable elements, plasmids | 14 | 0.3 | 26 | 0.6 |
| G    | Membrane transport | 231 | 5.7 | 251 | 5.8 |
| H    | Iron acquisition and metabolism | 60 | 1.5 | 102 | 2.4 |
| I    | RNA metabolism | 224 | 5.5 | 207 | 4.8 |
| J    | Nucleosides and nucleotides | 115 | 2.8 | 128 | 3.0 |
| K    | Protein metabolism | 279 | 6.8 | 289 | 6.7 |
| L    | Cell division and cell cycle | 33 | 0.8 | 33 | 0.8 |
| M    | Morality and chemotaxis | 120 | 2.9 | 137 | 3.2 |
| N    | Regulation and cell signaling | 126 | 3.1 | 139 | 3.2 |
| O    | Secondary metabolism | 6 | 0.1 | 4 | 0.1 |
| P    | DNA metabolism | 110 | 2.7 | 120 | 2.8 |
| Q    | Fatty acids, lipids, and isoprenoids | 199 | 4.9 | 190 | 4.4 |
| R    | Nitrogen metabolism | 50 | 1.2 | 50 | 1.2 |
| S    | Dornancy and sporulation | 5 | 0.1 | 3 | 0.1 |
| T    | Respiration | 148 | 3.6 | 128 | 3.0 |
| U    | Stress response | 195 | 4.8 | 199 | 4.6 |
| V    | Metabolism of aromatic compounds | 101 | 2.5 | 130 | 3.0 |
| W    | Amino acids and derivatives | 707 | 17.3 | 728 | 16.9 |
| X    | Sulfur metabolism | 59 | 1.4 | 103 | 2.4 |
| Y    | Phosphorus metabolism | 45 | 1.1 | 79 | 1.8 |
| Z    | Carbohydrates | 423 | 10.4 | 480 | 11.1 |

**Table 5** Secondary metabolite clusters identified in Pseudomonas sp. H3 with antiSMASH5.0.

| Cluster | Type | From | To | Most similar known cluster | Similarity | MIBiG BGC-ID |
|---------|------|------|----|-----------------------------|-----------|--------------|
| 1       | Resorcinol | 1 | 32,672 | Pyoverdine | 12 % | BGC0000413 |
| 2       | NRPS-like | 1 | 20,451 | Mangotorxin | 71 % | BGC0000387 |
| 3       | Hesperalone | 60,840 | 81,502 | – | – | – |
| 4       | Siderophore | 117,806 | 129,707 | – | – | – |
| 5       | Arylpolyene | 2,428 | 46,051 | APE VF | 40 % | BGC0000837 |
| 6       | Bacteriocin | 16,482 | 28,126 | – | – | – |
| 7       | Bacteriocin | 50,089 | 60,982 | – | – | – |
| 8       | Betalactone | 12,457 | 35,644 | Fengycin | 13 % | BGC0001095 |
| 9       | Siderophore | 260,231 | 272,153 | – | – | – |
| 10      | NRPS | 131,179 | 182,321 | Pseudomonine | 100 % | BGC0000410 |
| 11      | Ectoine | 225,551 | 235,937 | – | – | – |
| 12      | transAT-PKS,NRPS | 245,969 | 338,222 | Leinamycin | 8 % | BGC0001101 |
fluorescent, which harmonizes the observation of strain RGB on CSGA medium.

The siderophore production was tested in 5 and 100 ml scale with the glucose as sole carbon source. Therefore, precultures were grown for three days in LB medium (30 °C, 160 rpm), harvested by centrifugation (10,000 × g), washed twice with sterile saline, and re-suspended in 10 % of the initial volume with sterile saline. The main culture was inoculated 1:50 with cell suspension. To produce siderophores, a M9 minimal medium with low phosphate content was chosen containing 1.28 g l⁻¹ Na₂HPO₄, 0.3 g l⁻¹ KH₂PO₄, 0.5 g l⁻¹ NaCl, 1 g l⁻¹ NH₄Cl and 10 ml l⁻¹ goodie mix. Goodie mix solution consists of 385 mM MgSO₄, 10 mM CaCl₂, 0.1 mM thiamine and 125 ml l⁻¹ trace element solution (49 g l⁻¹ MgCl₂, 2 g l⁻¹ CaCl₂, 1.44 g l⁻¹ ZnSO₄·7H₂O, 0.85 g l⁻¹ MnSO₄·H₂O, 0.24 g l⁻¹ CuSO₄·5 H₂O, 0.06 g l⁻¹ H₃BO₃, 51 ml l⁻¹ HCl). 20 mM glucose was added as substrate. All glassware used for siderophore production was washed with 6 M HCl in order to remove iron [35].

The highest siderophore production was determined after 3 days with about 130 μM* (°(desferrioxamine B) equivalent [14]) in 5 ml scale. This is in a similar range compared to P. aeruginosa strains, although it has to be mentioned that the cultivation conditions are slightly different and the concentration was determined already after 1 day [36,37] (Table 10). Further, it can be seen that the siderophore concentration is lower in a bigger cultivation volume. Binding of other metal ions (Al³⁺, Ga³⁺ and Cu²⁺) was determined in the culture supernatant by adapted CAS-assays (Fig. 4) [14]. All of the tested metal ions can be chelated by the siderophores. This is in accordance with previous findings on pyoverdin and pyochelin [38]. For pseudomonine no other ligands than iron have been tested so far.

### Table 6
Pseudomonine related gene cluster in Pseudomonas sp. H3.

| Locus | Gene   | Description                                      | Accession  | % ID | % Coverage |
|-------|--------|--------------------------------------------------|------------|-----|------------|
| 4876  | –      | Ferrichrome-iron receptor                        | –          | –   | –          |
| 4877  | orf10  | Iron compound ABC uptake transporter substrate-binding protein | ARS0193 | 84  | 76         |
| 4878  | orf9   | Ferric siderophore ABC transporter, ATP-binding protein | ARS0192 | 89  | 100        |
| 4879  | orf8   | Iron compound ABC uptake permease protein PucC | ARS0191 | 85  | 95         |
| 4880  | orf7   | Iron compound ABC transporter, permease protein  | ARS0190 | 95  | 100        |
| 4881  | orf6   | Putative ABC transporter permease/ATP-binding protein | ARS0189 | 87  | 100        |
| 4882  | orf5   | Putative ABC transporter permease/ATP-binding protein | ARS0188 | 87  | 100        |
| 4883  | orf4   | Sigma factor, ECF-superfamily                     | ARS0187 | 81  | 98         |
| 4884  | pmsG   | NRPS                                            | ARS0186 | 79  | 100        |
| 4885  | pmsF   | N-hydroxylase                                    | ARS0185 | 87  | 73         |
| 4886  | hyp    | Hypothetical protein                             | –          | –   | –          |
| 4887  | orfD   | NRPS                                            | ARS0184 | 82  | 100        |
| 4888  | pmsC   | Isochoromate synthase (EC 5.4.4.2)                | CAA7028 | 86  | 100        |
| 4889  | pmsE   | NRPS                                            | CAA7029 | 90  | 100        |
| 4890  | pmsA   | Pyridoxal-dependent histidine decarboxylase      | CAA7030 | 86  | 97         |
| 4891  | pmsB   | Isochoromate pyruvate-lyase (EC 4.2--4)           | CAA7031 | 76  | 100        |

### Table 7
Secondary metabolite clusters identified in Pseudomonas sp. RGB with antiSMASH5.0.

| Cluster | Type   | From | To   | Most similar known cluster | Similarity | MIBiG BGC-ID |
|---------|--------|------|------|------------------------------|------------|--------------|
| 1       | NRPS   | 33,192 | 100,383 | Pyoverdin                    | 10 %       | BGC0000413   |
| 2       | NRPS   | 48,274 | 100,804 | Pyochelin                    | 100 %      | BGC0000412   |
| 3       | Phentazine,hectoractone | 196,591 | 219,395 | Pyocyanine                  | 100 %      | BGC0000936   |
| 4       | Thiopeptide | 433,489 | 463,427 | Lipopolysaccharide          | 5 %        | BGC0000774   |
| 5       | Aspyloxyene | 304,851 | 348,426 | APE Vf                      | 45 %       | BGC0000837   |
| 6       | NRPS   | 135,021 | 186,674 | Viscosin                    | 75 %       | BGC0001312   |
| 7       | NAGCN  | 85,373 | 100,323 | –                            | –          | –            |
| 8       | NRPS   | 133,328 | 186,224 | Pyoverdin                   | 10 %       | BGC0000413   |
| 9       | Bacteriocin | 593,687 | 604,532 | –                            | –          | –            |
| 10      | Bacteriocin | 417,217 | 428,095 | –                            | –          | –            |
| 11      | Betalactone | 179,482 | 202,682 | Fungycin                    | 13 %       | BGC0001095   |
| 12      | NRPS-like | 1    | 21,957 | Mangatoxin                  | 71 %       | BGC0000387   |

[35] M. Hofmann et al., Biotechnology Reports 25 (2020) e00403
Table 8
Pyochelin related gene cluster in Pseudomonas sp. RGB.

| Locus | Gene | Description                                              | Accession | % ID | % Coverage |
|-------|------|----------------------------------------------------------|-----------|------|------------|
| 3845  | pchA | Menaquinone-specific isochorismate synthase (EC 5.4.4.2) | CAA57969  | 44   | 99         |
| 3846  | pchB | Isochorismate pyruvate-lyase (EC 4.2.99.21)              | CAA57968  | 61   | 100        |
| 3847  | pchC | Pyochelin biosynthetic protein PchC, predicted thioesterase | CAA57967  | 62   | 92         |
| 3848  | pchD | 2,3-Dihydroxybenzoate-AMP ligase (EC 2.7.7.58)           | CAA57966  | 71   | 95         |
| 3849  | pchR | Transcriptional regulator PchR                           | NP_252917 | 71   | 100        |
| 3850  | pchE | NRPS                                                     | AAC83656  | 59   | 100        |
| 3851  | pchF | NRPS                                                     | AAC83657  | 62   | 100        |
| 3852  | pchG | Thiazolinyl imide reductase                              | AA01463   | 61   | 99         |
| 3853  | pchH | Putative ABC iron siderophore transporter                 | AA01464   | 54   | 99         |
| 3854  | pchI | Putative ABC iron siderophore transporter                 | AA01462   | 55   | 100        |
| 3855  | fptA | Fe(III)-pyochelin outer membrane receptor                | AA43215   | 74   | 97         |
| 3856  | fptB | Hypothetical protein in pyochelin cluster                | AA43214   | 58   | 21         |
| 3857  | fptC | Putative iron-regulated membrane protein                 | AA43215   | 51   | 72         |
| 3858  | fptX | Inner-membrane permease                                  | NP_252908 | 67   | 93         |

Table 9
Pyoverdine related gene clusters in Pseudomonas sp. RGB.

| Locus | Gene | Description                                              | Accession | % ID | % Coverage |
|-------|------|----------------------------------------------------------|-----------|------|------------|
| 1153  | pvdP | Tyrosinase                                               | AAX16288  | 65   | 96         |
| 1154  | pvdM | Periplasmic enzyme                                        | AAX16289  | 76   | 99         |
| 1155  | pvdN | Periplasmic enzyme                                        | AAX16290  | 62   | 100        |
| 1156  | pvdO | Periplasmic enzyme                                        | AAX16291  | 75   | 93         |
| 1157  | pvdF | N5-hydroxyornithine transformylase                       | AAX16292  | 80   | 99         |
| 1158  | pvdE | ABC transporter (secretion)                              | AAX16293  | 78   | 100        |
| 1159  | fbaA | Ferripyoverdine receptor protein                         | AAX16294  | 65   | 98         |
| 1160  | pvdD | NRPS                                                    | AAX16295  | 54   | 99         |
| 1161  | pvdJ | NRPS                                                    | AAX16296  | 48   | 97         |
| 1162  | pvdL | NRPS                                                    | AAX16297  | 56   | 100        |
| 2550  | mbtH | MbtH-like NRPS chaperone                                  | AAG05800  | 85   | 95         |
| 2551  | pvdH | Aminotransferase                                         | AAG05801  | 85   | 97         |
| 2560  | pvdL | NRPS                                                    | AAG05812  | 74   | 100        |
| 2561  | pvdG | Thioesterase                                            | AAG05813  | 53   | 98         |
| 2562  | pvdS | ECF sigma factor                                         | AAG05814  | 92   | 93         |
| 4143  | pvdQ | Ntn-type hydrolase                                       | AAG05873  | 54   | 98         |
| 5639  | qpmQ | Outer membrane pyoverdine eflux protein                  | AAG05779  | 63   | 97         |
| 5640  | pvdF | Pyoverdine eflux carrier and ATP binding protein         | AAG05778  | 81   | 98         |
| 5641  | pvdR | Pyoverdine eflux specific eflux protein                  | AAG05777  | 72   | 99         |
| 5642  | fpvI | ECF sigma factor                                         | AAG05775  | 72   | 99         |
| 5643  | pvdA | L-ornithine N5-oxygenase                                 | AAG05774  | 75   | 99         |
Table 10
Siderophore production of strain H3 and RGB on glucose in 5 ml and 100 ml scale.

| Strain                  | Scale in ml | Carbon source | Siderophore production in μM After x days | References |
|------------------------|-------------|---------------|------------------------------------------|------------|
| Pseudomonas sp. H3     | 5           | 20 mM Glucose | 135°                                     | This study |
|                        | 100         | 20 mM Glucose | 80°                                      |            |
| Pseudomonas sp. RGB    | 5           | 20 mM Glucose | 130°                                     | This study |
|                        | 100         | 20 mM Glucose | 70°                                      |            |
| P. aeruginosa FP6      | 100         | 56 mM Glucose | 20                                       | n.s. [37]  |
|                        |             | 34 mM Succinate | 125                                      | 1.5        |
| P. aeruginosa PSS      | 3500        | 56 mM Glucose | 180                                      | 1.25 [36]  |
|                        |             | 25 mM Succinate | 60                                       |            |
|                        |             | 7 mM Glutamic acid | 140                                      | 1         |

n.s. – not specified.
° Calculated in desferrioxamine B equivalents according to [14].

Fig. 4. Binding of the metal ions Al³⁺, Ga³⁺ and Cu²⁺ by culture supernatants of Pseudomonas sp. H3 and RGB. DFOBeq. – desferrioxamine B equivalents.

Author contribution
MH isolated the strains. MH, VS and SH performed the strain characterization including growth experiments and siderophore production. MH and TH performed the DNA isolation, genome sequencing, annotation and analyzes. TH, MH and DT wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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