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Two draft genome sequences of *Pseudomonas jessenii* strains isolated from a copper contaminated site in Denmark

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**Abstract**

*Pseudomonas jessenii* C2 and *Pseudomonas jessenii* H16 were isolated from low-Cu and high-Cu industrially contaminated soil, respectively. *P. jessenii* H16 displayed significant resistance to copper when compared to *P. jessenii* C2. Here we describe genome sequences and interesting features of these two strains. The genome of *P. jessenii* C2 comprised 6,420,113 bp, with 5814 protein-coding genes and 67 RNA genes. *P. jessenii* H16 comprised 6,807,788 bp, with 5995 protein-coding genes and 70 RNA genes. Of special interest was a specific adaptation to this harsh copper-contaminated environment as *P. jessenii* H16 contained a novel putative copper resistance genomic island (GI) of around 50,000 bp.

**Keywords:** *Pseudomonas jessenii*, Comparative genomics, Copper resistance

**Introduction**

Copper is an essential micronutrient in most organisms and required as a co-factor in biological processes such as redox reactions (electron transport, oxidative respiration, denitrification) [1, 2]. However, at higher concentrations copper will become toxic and inhibit or kill cells. Therefore, microorganisms have developed sophisticated copper homeostasis and resistance mechanisms in order to maintain the normal cellular copper supply to essential cuproenzymes while avoiding copper poisoning [3, 4]. Some highly copper resistant microorganisms have attracted great interests due to potential biotechnological applications in bio-mining and bioremediation of environments contaminated with copper [5].

*Pseudomonas* spp. are ubiquitous inhabitants of soil, water and plant surfaces belonging to the *Gammaproteobacteria*. *Pseudomonas* spp. has an exceptional capacity to produce a wide variety of secondary metabolites, including antibiotics that are toxic to plant pathogens [6, 7]. *Pseudomonas jessenii* was also found to be an important rhizobacterium conferring protection against a number of soilborne plant pathogens [8]. *P. jessenii* C2 and *P. jessenii* H16 were isolated from low-Cu soil and high-Cu soil from an abandoned wood impregnation site in Hygum, Denmark, respectively [9]. The Hygum site was contaminated with copper sulfate from 1911 to 1924, then the area was cultivated until 1993 and has been a fallow field since then [9, 10]. *P. jessenii* H16 was able to grow in medium containing high concentrations of copper, whereas *P. jessenii* C2 was sensitive to high copper concentrations. Here, we present the genome sequences, a brief characterization and annotation of *P. jessenii* C2 and *P. jessenii* H16.

**Organism information**

**Classification and features**

A highly copper contaminated high-Cu soil and a corresponding low-Cu soil were collected (0–20 cm depth) from a well-described Cu gradient field site in Hygum, Denmark. The high-Cu site was contaminated almost exclusively with CuSO₄ more than 90 years ago [9]. The adjacent low-Cu control site was located just outside the contaminated area and had been subjected to the same land use for more than 80 years. The low-Cu and high-Cu soil had similar physicochemical characteristics except for their total Cu contents of 21 and 3172 mg kg⁻¹, respectively [9, 11]. Bacteria were isolated from replicated soil subsamples (*n* = 3) and diluted, spread-plated on *Pseudomonas*-selective Gould’s S1 agar [11]. For each dilution series, 30 colonies emerging after two days at 25 °C were selected and isolated in pure...
Culture by repeated plating [11]. Two of the resulting isolates were selected for further study. *P. jessenii* H16 was able to grow at high concentration of Cu (2 mM) on one-tenth strength LB agar, whereas *P. jessenii* C2 only grew with up to 0.125 mM Cu.

Strain C2 and H16 were both Gram-reaction negative. Cells of strain C2 and H16 were rod shaped with rounded ends and motile. The cells of C2 were 2.12–2.45 μm (mean, 2.28 μm) in length compared to 0.42–0.57 μm in size (Fig. 1a). The cells of H16 were 1.95–2.38 μm × 0.42–0.57 μm in size (Fig. 1b). No Sporulation was observed for both strains. The colonies were white and translucent on Gould’s S1 agar medium. Growth occurred aerobically at 4–37 °C, and optimal growth was observed at 30 °C, pH 7.0 for strain C2. Strain H16 preferred pH 6.7, at 30 °C for optimal growth. Both strains grew in 0–4 % (w/v) NaCl (Tables 1 and 2).

**Chemotaxonomy**

Fatty acid analyses were performed by the Identification Service of the DSMZ, Braunschweig, Germany [12]. The fatty acid profiles were similar when comparing strains C2 and H16. The major fatty acids of the two strains showed as follows: C₁₆ : 1ω7c and/or iso-C₁₅ : 0 2-OH (36.4 % in *P. jessenii* C2 and 40.1 % in *P. jessenii* H16); C₁₈ : 1ω7c (15.3 % in *P. jessenii* C2 and 10.8 % in *P. jessenii* H16) and C₁₆ : 0 (28.8 % in *P. jessenii* C2 and 34.6 % *P. jessenii* H16).

Biochemical properties were tested using API 20NE (BioMérieux) for Strains C2 and H16. In the API 20NE system, positive reactions for both strains were observed for nitrate reduction and production of arginine dihydrolase; negative reactions were observed for indole production, urease activity, Lysine and ornithine decarboxylase and gelatin hydrolysis (Additional file 1: Table S1). Strain C2 assimilated d-glucose, d-melibiose, d-sucrose, d-mannitol, l-rhamnose, inositol, trehalose, d-lyxose, l-arabinose and inositol as carbon sources, but not, l-rhamnose and sorbitol (Additional file 1: Table S1).

**16S rRNA gene analysis**

Comparative 16S rRNA gene sequence analysis using the EzTaxon database [13] indicated that strain C2 and H16 were both most closely related to *P. jessenii* CIP 105275T (GenBank accession no. AF068259) with sequence similarities of 99.87 and 99.14 %, respectively. Phylogenetic analysis was performed using the 16S rRNA gene sequences of strains C2, H16 and related species. Sequences were aligned and phylogenetic trees were constructed using Maximum Likelihood method implemented in MEGA version 6 [14]. The resultant tree topologies were evaluated by bootstrap analyses with 1000 random samplings (Fig. 2).

**Genome sequencing information**

**Genome project history**

Next-generation shotgun-sequencing was performed at the Beijing Genomics Institute (BGI, Shenzhen). The whole genome shotgun project of *P. jessenii* C2 and *P. jessenii* H16 has been deposited at DDBJ/EMBL/GenBank under the accession numbers JSAK00000000 and JSAL00000000. The version described in this paper is the first version. A summary of the project and the Minimum Information about a Genome Sequence [15] are shown in Table 3.

**Growth conditions and genomic DNA preparation**

*P. jessenii* C2 and *P. jessenii* H16 were aerobically cultivated on *Pseudomonas*-selective Gould’s S1 agar at 28 °C [16]. Total genomic DNA was extracted using Puregene Yeast/Bact Kit according to the manufacturer’s instructions (QIAGEN). The quantity of the genomic DNA was determined by Qubit® fluorometer (Invitrogen, CA, USA) with Qubit dsDNA BR Assay kit (Invitrogen, CA, USA) and amounted to 55 ng/μL of DNA for *P. jessenii* C2 and 48.2 ng/μL of DNA for *P. jessenii* H16.

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**Fig. 1** Micrograph of *Pseudomonas jessenii* C2 and H16 obtained by scanning electron microscopy. **a** *Pseudomonas jessenii* C2. **b** *Pseudomonas jessenii* H16
The genome sequencing of *P. jessenii* H16 and *P. jessenii* C2 was determined by BGI using the Illumina Hiseq2000 with a 500 bp library constructed [17], generating 1.09 gigabytes of DNA sequence with an average coverage of ~160 fold and ~170 fold; yielding 1,205,9244 and 1,203,8756 paired-end reads with a 90-bp read length, respectively. The resulting sequence data was quality assessed, trimmed, and assembled de novo as described previously [18] using CLCBio Genomic Workbench 7.0 (CLCBio, Denmark). *P. jessenii* H16 generated 78 contigs with an n50 value of 279,014 bp. *P. jessenii* C2 generated 64 contigs with an n50 value of 224,893 bp.
Genome annotation
The genes in the assembled genome were predicted based on the RAST database [19]. The predicted ORFs were annotated by searching clusters of orthologous groups [20] using the SEED database [21]. RNAmer 1.2 [22] and tRNAscanSE 1.23 [23] were used to identify rRNA and tRNA genes, respectively.

Genome properties
P. jessenii C2 contained 6,420,113 bp with a G+C content of 59.83%, 5881 predicted genes, 5814 were protein-coding genes, 63 tRNA genes and 4 rRNA genes. In total, 5179 genes were assigned to biological functions and 635 were annotated as hypothetical proteins. P. jessenii H16 contained 6,807,788 bp, with a GC content of 59.02%.

Table 3 Project information

| MIGS ID | Property                          | Term                        |
|---------|----------------------------------|-----------------------------|
| MIGS 31 | Finishing quality                | High-quality draft          | High-quality draft          |
| MIGS-28 | Libraries used                   | One paired-end Illumina library | One paired-end Illumina library |
| MIGS 29 | Sequencing platforms             | Illumina HiSeq 2000         | Illumina HiSeq 2000         |
| MIGS 31.2| Fold coverage                    | 170x                        | 160x                        |
| MIGS 30 | Assemblers                       | CLC Genomics                | CLC Genomics                |
|         |                                  | Workbench, version 7.0.4    | Workbench, version 7.0.4    |
| MIGS 32 | Gene calling method              | Glimmer 3.0                 | Glimmer 3.0                 |
|         | Locus Tag                        | NL64                        | RY26                        |
|         | GenBank ID                        | JSAK00000000.1               | JSAL00000000.1               |
|         | GenBank Date of Release           | 2014/12/17                  | 2014/12/17                  |
|         | GOLD ID                          | Gp0157184                    | Gp0157185                    |
|         | BIOPROJECT                       | PRJNA264019                  | PRJNA264019                  |
| MIGS 13 | Source Material Identifier       | HC-Cu2                      | HC_Cu16                      |
|         | Project relevance                | Low-Cu soil                 | Copper contaminated soil     |
### Table 4: Genome statistics

| Attribute               | P. jessenii C2                | % of total | P. jessenii H16                | % of total |
|-------------------------|------------------------------|------------|------------------------------|------------|
| Genome size (bp)        | 6,420,113                    | 100.00     | 6,807,788                    | 100.00     |
| DNA coding (bp)         | 5,484,120                    | 85.42      | 5,835,906                    | 85.72      |
| DNA G+C (bp)            | 3,851,154                    | 59.83      | 4,017,956                    | 59.02      |
| DNA scaffolds           | 64                           | -          | 78                           | -          |
| Total genes             | 5881                         | 100.00     | 6065                         | 100.00     |
| Protein coding genes    | 5814                         | 98.86      | 5995                         | 98.85      |
| RNA genes               | 67                           | 1.14       | 70                           | 1.15       |
| Pseudo genes            |                              |            |                              |            |
| Genes with function prediction | 5179 | 88.06 | 5344 | 88.11 |
| Genes assigned to COGs  | 4314                         | 73.75      | 4354                         | 71.79      |
| Genes with Pfam domains | 3595                         | 61.13      | 3587                         | 59.14      |
| Genes with signal peptides | 510  | 8.67      | 537                          | 8.85       |
| Genes with transmembrane helices | 1260 | 21.42 | 1343 | 22.14 |
| CRISPR repeats          | 38                           | -          | 11                           | -          |

### Table 5: Number of genes associated with general COG functional categories

| Code | P. jessenii C2 | %a | P. jessenii H16 | %a | Description |
|------|----------------|----|----------------|----|-------------|
| J    | 183            | 3.14         | 186           | 3.10         | Translation, ribosomal structure and biogenesis |
| A    | 1              | 0.02         | 2             | 0.03         | RNA processing and modification |
| K    | 425            | 7.31         | 425           | 7.09         | Transcription |
| L    | 147            | 2.53         | 135           | 2.25         | Replication, recombination and repair |
| B    | 2              | 0.34         | 3             | 0.05         | Chromatin structure and dynamics |
| D    | 35             | 0.60         | 35            | 0.58         | Cell cycle control, Cell division, chromosome partitioning |
| V    | 59             | 1.01         | 57            | 0.95         | Defense mechanisms |
| T    | 368            | 6.33         | 389           | 6.49         | Signal transduction mechanisms |
| M    | 239            | 4.11         | 282           | 4.70         | Cell wall/membrane biogenesis |
| N    | 128            | 2.20         | 135           | 2.25         | Cell motility |
| U    | 119            | 2.05         | 128           | 2.14         | Intracellular trafficking and secretion |
| O    | 175            | 3.01         | 168           | 2.80         | Posttranslational modification, protein turnover, chaperones |
| C    | 312            | 5.37         | 278           | 4.64         | Energy production and conversion |
| G    | 219            | 3.77         | 247           | 4.12         | Carbohydrate transport and metabolism |
| E    | 515            | 8.86         | 497           | 8.29         | Amino acid transport and metabolism |
| F    | 85             | 1.46         | 99            | 1.65         | Nucleotide transport and metabolism |
| H    | 177            | 3.04         | 193           | 3.22         | Coenzyme transport and metabolism |
| I    | 237            | 4.08         | 194           | 3.24         | Lipid transport and metabolism |
| P    | 300            | 5.16         | 286           | 4.77         | Inorganic ion transport and metabolism |
| Q    | 142            | 2.44         | 129           | 2.15         | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 532            | 9.15         | 572           | 9.54         | General function prediction only |
| S    | 444            | 7.64         | 451           | 7.52         | Function unknown |
| -    | 970            | 16.68        | 1104          | 18.42        | Not in COGs |

*aThe total is based on the total number of protein coding genes in the genome*
6065 predicted genes, and 5995 were protein-coding genes, 65 tRNA and 5 rRNA genes. Among the protein coding genes 5344 were assigned to biological functions, while 651 were annotated as hypothetical proteins. The properties and statistics of those two genomes are summarized in Table 4. The distribution of genes into COG functional categories is presented in Table 5 and Fig. 3.

**Insights into the genome**

Genes conferring resistances to heavy metals in the two studied strains are listed in Table 6. Copper efflux from the cytosol is mediated by the P₁B-type ATPase family, which is highly conserved from bacteria to humans [24]. Both *P. jessenii* C2 and *P. jessenii* H16 contained genes encoding a copper-transporting P₁B-type ATPase (CopA) with conserved CPCALG motif [25], a copper-responsive metalloregulatory protein CueR, and the multicopper oxidase CueO. In addition, one additional gene encoding a Cu⁺-ATPase is present on the genome of *P. jessenii* H16 as part of the GI discussed later. *P. jessenii* H16 also contained *ccol* encoding a Cu⁺-ATPase catalyzing a slower rate of efflux for copper insertion into cytochrome c oxidase [26]. The presence of a *cop* operon, comprising *copABCDRS* had been reported in related *P. fluorescens* SBW25 and *P. putida* KT2440 [27, 28]. Both *P. jessenii* strains contained *copCDRS* probably encoding proteins

![Circular map of the chromosome of *P. jessenii* C2 and *P. jessenii* H16. From outside to the center: *P. jessenii* H16 genes on forward strand (color by COG categories), *P. jessenii* H16 CDS on forward strand, tRNA, rRNA, other; *P. jessenii* H16 CDS on reverse strand, *P. jessenii* H16 tRNA, rRNA, other, genes on reverse strand (color by COG categories); *P. jessenii* C2 CDS blast with *P. jessenii* H16 CDS; *P. fluorescens* SW25 (NC_012660) CDS blast with *P. jessenii* H16 CDS; *P. jessenii* H16 GC content; *P. jessenii* H16 GC skew, where green indicates positive values and magenta indicates negative values.](image-url)
Table 6 P. jessenii C2 and P. jessenii H16 genes related to heavy metal resistance

| P. jessenii C2 | P. jessenii H16 | Predicted function |
|----------------|-----------------|--------------------|
| Protein id | Size/aa | Protein id | Size/aa | |
| KII28258 | 513 | KII28679 | 459 | Multicopper oxidase Cue-O-1 |
| KII31612 | 122 | KII28987 | 121 | Copper resistance protein CopC |
| KII31613 | 282 | KII28988 | 286 | Copper resistance protein CopD-1 |
| KII30013 | 133 | KII32596 | 138 | Cu(I)-responsive transcriptional regulator CopR |
| KII30014 | 798 | KII32595 | 798 | Copper-translocating P-type ATPase CopA-1 |
| KII30016 | 66 | KII32593 | 66 | Copper resistance protein CopZ |
| KII37329 | 149 | KII32956 | 149 | Metal-binding protein CopG-1 |
| KII33434 | 179 | KII28041 | 179 | Copper tolerance protein |
| KII33435 | 227 | KII28042 | 227 | Copper response regulator CusR-1 |
| KII33436 | 450 | KII28043 | 450 | Copper sensor histidine kinase CusS-1 |
| KII34384 | 759 | KII35062 | 770 | Lead, cadmium, zinc and mercury transporting ATPase |
| KII29504 | 116 | KII36460 | 428 | Arsenical pump membrane protein ArsB |
| KII37329 | 149 | KII36598 | 116 | Arsenical resistance operon repressor ArsR |
| KII37330 | 122 | KII36597 | 144 | Mercury transport protein MerC |
| KII30016 | 66 | KII36596 | 91 | Mercury transporter MerR |
| KII37329 | 122 | KII36595 | 116 | Mercuric transport protein MerT |
| KII30016 | 66 | KII36594 | 116 | Mercuric reductase MerA |
| KII37329 | 122 | KII36593 | 116 | Alkymercury lyase MerB |

P. jessenii H16 contained an additional putative metal fitness/pathogenicity island when compared with P. jessenii C2. It encompasses about 50,000 bp beginning at a gene encoding a sulfur carrier protein (KII37703) and ending with genes encoding Tn7 transposition proteins (KII37740-KII37743). This potential pathogenicity/fitness island harbored several copper resistance determinants including the cus determinant encoding CusABCRS (KII37706-37708, KII37711-37712) involved in periplasmic copper detoxification [32, 33]. In addition, genes encoding the P-type ATPase CopA, the multicopper oxidase CueO and CopBDG (KII37893, KII37715, KII37716, KII37709, KII37717) could be identified (Fig. 4). We also predicted specific GI for both P. jessenii H16 and P. jessenii C2 using the IsfindViewer [34]. Based on the automatic prediction algorithm two putative regions (coordinates KII37706-KII37717, KII37721-KII37737) were only identified in P. jessenii H16. Similar copper fitness islands could also be detected in P. extremaustralis 14-3b (AHIP00000000), isolated from a temporary pond in Antarctica; Pseudomonas sp.Ag1 (AKVH00000000) isolated from midguts of mosquitoes and P. fluorescens FH4 (AOHN00000000) [35–37]. This island also contained genes encoding the nickel efflux transporter NcrA (KII37721) and the transcriptional repressor NcrB (KII37723) [38]. Moreover, genes merTR-CAB (KII37733-37737) encoding a mercury-resistance determinant are present on this island [39]. Many of the various putative GI contain functions related to mobility such as integrases or mobile genetic elements (MGE) which includes transposons and IS elements. As shown in P. jessenii H16, these putative GI have conferred this strain with additional heavy metal resistance capability, which may be transferred to other bacteria via Tn7 transposons and are highly relevant for adaptation to this specific copper contaminated niche.

Conclusion

The draft genome sequences of P. jessenii C2 isolated from low-Cu soil and P. jessenii H16 isolated from high-Cu soil were determined and described here. H16 provided an insight into the genomic basis of the observed higher copper resistance when compared with C2. Based on analysis and characterization of the genome, P. jessenii H16 is predicted to be resistant to a number of heavy metals.
metal(loid)s, such as Hg$^{2+}$, Ni$^{2+}$, Cr$^{3+}$ and As$^{3+}$. Comparative genomic analysis of those two strains suggested acquisition of a fitness island encoding numerous genes involved in conferring resistance to Cu and other metals as an important adaptive mechanism enabling survival of \textit{P. jessenii} H16 in its Cu contaminated habitat. Possibly, \textit{P. jessenii} H16 may have potential for bioremediation of copper contamination environments.

Additional file

Additional file 1: Table S1. Phenotypic characteristics of C2, H16 and phylogenetically related \textit{P. jessenii} CIP 105275T. (DOCX 59 kb)

Abbreviations

BGI: Beijing Genomics Institute; GI: Genomic island; MGE: Mobile genetic elements

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Authors’ contributions

YQ drafted the manuscript, performed laboratory experiments, and analyzed the data; DW analyzed data; KKB isolated bacteria and assisted in selection of strains, planning and manuscript preparation; CR organized the study and drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

References

1. Chaturvedi KS, Henderson JP. Pathogenic adaptations to host-derived antibacterial copper. Front Cell Infect Microbiol. 2014;3.
2. González-Guerrero M, Raimunda D, Cheng X, Argüello JM. Distinct functional roles of homologous Cu$^{+}$ efflux ATPases in \textit{Pseudomonas aeruginosa}. Mol Microbiol. 2010;78(5):1246–58.
3. Fu Y, Chang FMJ, Giedroc DP. Copper transport and trafficking at the host-bacterial pathogen interface. Accounts Chem Res. 2014;47(12):3605–13.
4. Porcheron G, Garénaux A, Proulx J, Sabri M, Dozois CM. Iron, copper, zinc, and manganese transport and regulation in pathogenic \textit{Enterobacteria}: correlations between strains, site of infection and the relative importance of the different metal transport systems for virulence. Front Cell Infect Microbiol. 2013;3:90.
5. Navarro CA, Bernath-von D, Jerez CA. Heavy metal resistance strategies of acidophilic bacteria and their acquisition: importance for biomining and bioremediation. Biol Res. 2013;46(4):363–71.
6. Haas D, Keel C. Regulation of antibiotic production in root-colonizing \textit{Pseudomonas} spp. and relevance for biological control of plant disease. Annu Rev Phytopathol. 2003;41:117–53.
7. Raaijmakers JM, Vlami M, de Souza JT. Antibiotic production by bacterial biocontrol agents. Antonie Van Leeuwenhoek. 2002;81:537–47.
8. Deora A, Hatano E, Tahara S, Hashidoko Y. Inhibitory effects of furanone metabolites of a rhizobacterium, \textit{Pseudomonas jessenii}, on phytopathogenic

![Fig. 4 Putative copper fitness/pathogenicity island in \textit{P.jessenii} H16. Model of encoded proteins involved in copper resistance. CusA copper transporter, CusB RND transporter, CusC RND efflux outer membrane protein, CopD copper resistance protein, CusS-2 copper sensor histidine kinase, CusR-2 copper response regulator, CopA-2 copper-translocating P-type ATPase, CueO-2 multicopper oxidase, CopB copper resistance protein, CopG-2 metal-binding protein, CzcD cation transporter, B blue (type1) copper domain-containing protein CinA, H hypothetical protein, M putative metal-binding protein, Z copper chaperone](image-url)
