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GENOME ANNOUNCEMENT

Genome Sequence of the Polyhydroxybutyrate Producer *Pseudomonas extremaustralis*, a Highly Stress-Resistant Antarctic Bacterium

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***Pseudomonas extremaustralis* 14-3b presents genes involved in the synthesis of different polyhydroxyalkanoates, in tolerance and degradation of pollutants, and in microaerobic metabolism. Several genomic islands were detected. Genetic machinery could contribute to the adaptability to stressful conditions. This is the first genome sequence reported from a *Pseudomonas* isolated from cold environments.**

Pseudomonas extremaustralis 14-3^T was isolated from a temporary pond in Antarctica (8). This strain shows high heat, cold, and oxidative stress resistance (3, 4) in association with accumulation of large amounts of polyhydroxybutyrate (PHB), a type of polyhydroxyalkanoate (PHA). This polymer is synthesized from octanoate but not from glucose (1). Here, we report the genome sequence of *P. extremaustralis* 14-3b (DSM 25547), a natural derivative of the type strain able to produce PHB from both octanoate and glucose.

The sequence was obtained using a whole-genome shotgun strategy with a Roche 454 GS Titanium pyrosequencer at INDEAR, Argentina. Assembly was done by using Newbler version 2.5.3 with the -urt option with 20× genome coverage, generating 113 large contigs. The draft genome was 6,587,033 bases in length, with a mean GC content of 60.66%. Genome annotation was done using the standard operating procedures for prokaryotic annotation from the RAST server (5). A total of 5,934 coding sequences (CDS) and 62 structural RNAs (49 tRNAs) were predicted. Annotation covered 500 RAST subsystems (49%), while 1,516 CDS (26%) were classified as encoding hypothetical proteins.

The *P. extremaustralis* genome showed a well-developed antioxidant defense system that include five catalases, one superoxide dismutase, one cytochrome c_{551} peroxidase, and two alkyl hydroperoxide reductases. This strain is able to use nitrate as an alternative electron acceptor (9); in concordance, its genome was found to have 18 genes related to nitrate metabolism. Genes involved in nitrate, nitric oxide, and nitrous oxide reduction were present. Genes that code for nitrite reductases, with the exception of *nirM*, were not found, explaining its inability to denitrify (9). Additionally, 9 genes encoding enzymes involved in arginine and pyruvate fermentation were detected. Low-oxygen processes are controlled in *Pseudomonas* species by the global regulator Anr (6). PRODORIC software and manual searching allowed the detection of 167 genes in the Anr regulon.

Genes that could allow *P. extremaustralis* to cope with toxic compounds like heavy metals (*copRSABCD*, *czcABCD*, and *znuABC*) and arsenic (*asrRBC* and the gene encoding the arsenical resistance protein ACR3) as well as phenol and alkane degradation pathways (*dmpKLMNOP* and *alkB*, respectively) were also present.

Whole-genome analysis using IslandViewer software (7) revealed the presence of 28 genomic islands (GI), some of them related to environmental adaptability. More relevant islands contained genes involved in osmotic resistance, vitamin biosynthesis, PHB and exopolysaccharide metabolism, and proteins related to transport systems. We have described that PHB biosynthesis genes are located in a GI (2). Genome analysis showed other three genes involved in PHA metabolism in the same GI. *Pseudomonas* species typically have the *phaC1ZC2DIF* cluster, which is responsible for synthesis of medium-chain-length PHA. In *P. extremaustralis*, these genes were also detected, but *phaI* and *phaF* were not located downstream of *phaD* due to the insertion of seven open reading frames with high similarity to those found in the betaproteobacteria.

The overall genome analysis revealed a high potential for adaptability to extreme environmental conditions.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number AHIP00000000. The version described in this paper is the first version, AHIP01000000.

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REFERENCES

1. **Ayub ND, Pettinari MJ, Méndez BS, López NI.** 2006. Impaired polyhydroxybutyrate biosynthesis from glucose in *Pseudomonas* sp. 14-3 is due to a defective beta-ketothiolase gene. FEMS Microbiol. Lett. **264**:125–131.
2. **Ayub ND, Pettinari MJ, Méndez BS, López NI.** 2007. The polyhydroxyalkanoate genes of a stress resistant Antarctic *Pseudomonas* are situated within a genomic island. Plasmid **58**:240–248.
3. **Ayub ND, Pettinari MJ, Ruiz JA, López NI.** 2004. A polyhydroxybutyrate

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producing *Pseudomonas* sp. isolated from Antarctic environments with high stress resistance. Curr. Microbiol. **49**:170–174.
4. **Ayub ND, Tribelli PM, López NI.** 2009. Polyhydroxyalkanoates are essential for maintenance of redox state in the Antarctic bacterium *Pseudomonas* sp. 14-3 during low temperature adaptation. Extremophiles **13**:59–66.
5. **Aziz RK, et al.** 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics **9**:75.
6. **Galimand M, Gamper M, Zimmermann A, Haas D.** 1991. Positive FNR-like control of anaerobic arginine degradation and nitrate respiration in *Pseudomonas aeruginosa.* J. Bacteriol. **173**:1598–1606.
7. **Langille MGI, Brinkman FSL.** 2009. IslandViewer: an integrated interface for computational identification and visualization of genomic islands. Bioinformatics **25**:664–665.
8. **López NI, et al.** 2009. *Pseudomonas extremaustralis* sp. nov. a poly(3-hydroxybutyrate) producer isolated from an Antarctic environment. Curr. Microbiol. **59**:514–519.
9. **Tribelli PM, Méndez BS, López NI.** 2010. Oxygen sensitive global regulator, Anr, is involved in biosynthesis of poly-3-hydroxybutyrate (PHB) in *Pseudomonas extremaustralis.* J. Mol. Microbiol. Biotechnol. **19**:180–188.