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Complete genome sequence of *Marinobacter salarius* HL2708#2 isolated from a lava sea water environment on Jeju Island

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제주용암 해수 환경에서 분리한 *Marinobacter salarius* HL2708#2의 유전체 해독

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During screening of microbes for compounds having cosmetic benefits, we isolated *Marinobacter salarius* HL2708#2 from lava seawater on Jeju Island, Republic of Korea. The complete genome sequence was determined. Strain HL27080#2 features a circular chromosome of 4,304,603 bp with 57.21% G+C content and a 244,163 bp plasmid with 53.14% G+C. There were 4,180 protein coding sequences identified, along with 49 transfer RNA and 18 ribosomal RNA noncoding genes. The genome harbored genes for the utilization of alcohol, maltose/starch, and monosaccharide as sole carbon sources. Genes responsible for halophilic characteristics and heavy metal resistance could be annotated, as well as aromatic and alkane hydrocarbons. Contrary to the prior report that *M. salarius* is negative for nitrate and nitrite reduction, nitrate/nitrite reductase along with nitrate/nitrate transporters and nitronate monooxygenase were evident, suggesting that strain HL2708#2 may be able to denitrify extracellular nitroalkenes to ammonia.

Keywords: *Marinobacter salarius*, complete genome sequence, lava, seawater

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Marinobacter salarius strain HL2708#2 is a Gram-negative, rod-shaped, and chemoheterophic bacterium that was isolated from lava seawater on Jeju Island (Handong Lava, N 33.5327°, E 126.8204°) during our investigation of the cosmetic potential of marine microbial species (Koo *et al.*, 2018). Using 16S ribosomal RNA (rRNA) similarity and genome analysis, strain HL2708#2 was identified as *Marinobacter salarius* species (Koo *et al.*, 2018). The type species of this genus is *M. hydrocarbonoclasticus*, which was isolated as a halotolerant hydrocarbon-degrading bacterium (Gauthier *et al.*, 1992). Halotolerant *M. salarius* was previously isolated from a seawater sample contaminated by radionuclides collected from Chazhma Bay, Russia, near the East Sea of Korea (Ng *et al.*, 2014).

Here we report the complete genome of *M. salarius* strain HL2708#2 collected from an isolated lava seawater environment on Jeju Island. This isolate may differ from the Chazhma Bay strain R9SW1.

Genomic DNA was extracted using the i-genomic BYF mini kit (iNtRON Biotechnology) following the manufacturer's protocols. Genome sequencing of strain HL2708#2 was performed using PacBio RS II single-molecule real-time (SMRT) sequencing

technology (Pacific Biosciences). A standard PacBio library with inserts averaging 20 kb were prepared using 5 μg of genomic DNA and were sequenced, yielding over 113 × genome coverage. *De novo* assembly of the 113,192 reads with 7,066 nucleotides on average (799,861,839 bp total) was conducted using the hierarchical genome-assembly process pipeline of the SMRT analysis v2.3.0 (Chin *et al.*, 2013). To correct sequencing errors that can occur at both ends of a contig, the SMRT resequencing protocol was performed with an assembly in which the first half of the contig was switched with the second half.

Protein coding genes were predicted by Prodigal v.2.6.3 (Hyatt *et al.*, 2010). Signal peptides and transmembrane regions of predicted genes were predicted using SignalP v4.1 (Petersen *et al.*, 2011) and TMHMM v2.0 (Krogh *et al.*, 2001), respectively. BLAST searches were performed against UniProt (Wu *et al.*, 2006), Pfam (Finn *et al.*, 2014), and COG (Tatusov *et al.*, 2003) databases to functionally annotate the predicted genes. The rRNA, transfer RNA (tRNA), and miscellaneous features were predicted using Rfam v12.0 (Griffiths-Jones *et al.*, 2005). The antiSMASH web-based tool was used for the prediction of secondary metabolite biosynthesis gene clusters (Medema *et al.*, 2011). The graphical circular map of the complete genome was constructed and visualized using Circos v0.67 (Krzywinski *et al.*, 2009).

The genome was found to comprise a circular chromosome of 4,304,603 bp with 57.21% G+C content, and a 244,163 bp plasmid (pHL2708Z5) with 53.14% G+C content. A total of 4,241 genes were predicted in the genome. Of these, 4,180 are protein coding genes. The total length of coding regions is 4,145,862 bp. Putative functions were assigned to 2,720 of the protein coding genes and the remainder were annotated as hypothetical proteins. The genome also has 18 rRNA genes (three operons of 5S, 16S, and 23S) and 49 tRNA genes. The properties and the statistics of the genome are summarized in tables on the genome feature page and a COG functional category table on the gene prediction and annotation page.

Chemoheterotrophic *M. salarius* strain HL2708#2 harbors full gene sets for glycolysis via the embden-meyerhof-parnas pathway, with the production of pyruvate, the pentose phosphate pathway that produces NADPH and pentoses, as well as ribose 5-phosphate, tricarboxylic acid cycle and oxidative phosphorylation system.

The strain contains genes encoding maltose alpha-D-glucosyltransferase (MTMN5_03192) with the maltodextrin/maltose transport system (MTMN5_03188 to MTMN5_03190) and alpha-amylase (MTMN5_01691). Additional genes that were identified include those encoding acetyl-CoA synthetase from acetate to acetyl-CoA, aldehyde dehydrogenase, alcohol dehydrogenase 1/7, and alcohol dehydrogenase that converts ethanol to acetate (MTMN5_00558, MTMN5_01466, MTMN5_03642, and MTMN5_04050, respectively). These results imply that the strain is able to oxidize alcohol and utilize maltose/maltodextrin and starch as sole carbon sources as well as monosaccharides such as glucose and fructose. Amino acid-auxotrophic characteristics were not found.

The rod shape-determining mre*BCD* operon (MTMN5_02860 to MTMN5_02862) was identified with the genes encoding the rod shape-determining protein rod*A* and penicillin-binding protein2 (MTMN5_00727 and MTMN5_00728). Gene clusters involved in flagellar biosynthesis, assembly, and chemotaxis for motility were identified (MTMN5_02947 to MTMN5_02952, MTMN5_03001 to MTMN5_03021, MTMN5_03025 to MTMN5_03034, respectively). In addition, the gene clusters related to *sec*-independent protein translocation, type II general secretion pathway, and type VI secretion were identified (MTMN5_02272 to MTMN5_02274, MTMN5_03732 to MTMN5_03740, and MTMN5_01948 to MTMN5_01961, respectively). Genes responsible for halophilic characteristics were identified. These included Na^+/H^+ antiporter (MTMN5_02734 to MTMN5_02739), K^+ transporter, glycine betaine uptake system (MTMN5_00494 to MTMN5_00497), ectoine synthase (MTMN5_00497, MTMN5_03635, and MTMN5_04058), and 5-hydroxyectoine synthase (MTMN5_02464). Gene clusters related to heavy metal resistance were identified and included copper-resistance (MTMN5_00266 to MTMN5_00268) and cobalt/zinc/cadmium-resistance (MTMN5_04178 to MTMN5_04180). Two core biosynthetic gene sets for the synthesis of non-ribosomal peptides were identified, putatively including the pyoverdine siderophore (MTMN5_00679 to MTMN5_00680 and MTMN5_03394 to MTMN5_03395).

Marinobacter previously isolated from a hydrocarbon polluted site was found to degrade hydrocarbon. The strain was shown to harbor three gene clusters consisting of eight genes for catechol degradation. One cluster was located in the chromosome

(MTMN5_01390 to MTMN5_01397) and the other two were in a plasmid (MTMN5_04009 to MTMN5_04016 and MTMN5_04140 to MTMN5_04147). In addition, the gene clusters involved in the degradation of phenol and benzoate were identified (MTMN5_01399 to MTMN5_01404 and MTMN5_04135 to MTMN5_04138, respectively). Furthermore, two gene clusters with identical synteny composed of five genes were identified (MTMN5_03640 to MTMN5_03644 and MTMN5_04052 to MTMN5_04048). Four genes of the cluster were identified as alkane 1-monooxygenase, rubredoxin dehydrogenase, aldehyde dehydrogenase, and medium-chain-fatty-acid-CoA ligase. They are involved in the degradation of alkane. One gene contains the GMC oxidoreductase domain. Other genes essential for degrading alkane, including 3-phenylpropionate/

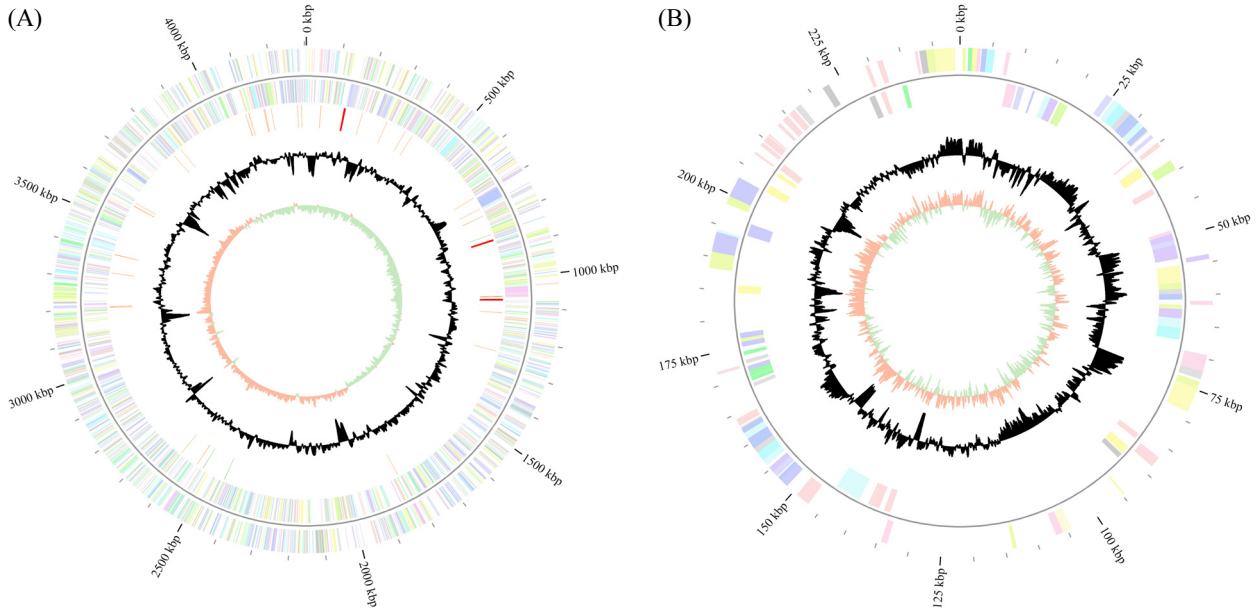

Fig. 1. Graphical circular map of the chromosome (A) the pHL2708Z5 (B) of HL2708#2. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs orange, rRNAs red, other RNAs green), GC content (black), and GC skew (light green/orange).

Table 1. General feature of genome

Attribute	Chromosome		pHL2708Z5	
	Value	% of Total	Value	% of Total
Genome size (bp)	4,304,603	100.00%	244,163	100.00%
Protein coding (bp)	3,938,379	91.49%	208,419	85.36%
DNA G+C (bp)	2,462,499	57.21%	129,751	53.14%
DNA scaffolds	1		1	
Total genes	3,983	100.00%	258	100.00%
Protein coding genes	3,922	98.47%	258	100.00%
RNA genes[a,b]	61	1.53%	0	0.00%
Genes with function prediction	2,610	65.53%	110	42.64%
Genes assigned to COGs	3,041	76.35%	130	50.39%
Genes with Pfam domains	3,307	83.03%	172	66.67%
Genes with signal peptides	409	10.27%	33	12.79%
Genes with transmembrane helices	1,032	25.91%	58	22.48%

[a] No. of rRNA operon: 3 copies
[b] No. of tRNA gene: 49 genes

trans-cinnamate dioxygenase ferredoxin reductase (MTMN5_03648) and rubredoxin-NAD (+) reductase (MTMN5_02109 and MTMN5_04054) were identified. Moreover, the strain contains genes encoding nitronate monooxygenase (MTMN5_00498, MTMN5_02514) and nitrilase (MTMN5_01287), which hydrolyze nitroalkene compounds to acetaldehyde compounds and nitrite and nitriles to carboxylic acids and ammonia, respectively. These results imply that the strain is an important decomposer that utilizes aromatic and alkane hydrocarbon as a carbon and energy source in hydrocarbon polluted sites, and degrades nitroalkane and nitrile compounds, such as 3-nitropropionic acid, benzonitrile, phenylpropionitrile, and others, which are common in legumes and fungi.

M. salaries was reported to be unable to reduce nitrate and nitrite (Ng *et al.*, 2014). However, we identified the presence of the genes responsible for the transport of extracellular nitrate/nitrite (MTMN5_01533, MTMN5_01534, MTMN5_01535), nitrate reductase (MTMN5_01531), and nitrite reductase (MTMN5_01537, MTMN5_01538, and MTMN5_01539). These results suggest that the strain is able to reduce extracellular nitrate/nitrite and nitrite producing from denitrification of nitroalkenes to ammonia by nitronate monooxygenase.

Nucleotide sequence and strain accession numbers

The genome sequence of *M. salarius* HL2708#2 was deposited at GenBank under the accession numbers CP021333 and CP021334. The strain is available from Cotde Inc. (daniel@cotde.co.kr).

적 요

향장원료 개선을 위한 미생물 탐색 실험을 통하여 *Marinobacter salarius* HL2708#2을 제주도의 용암 해수 환경에서 분리하였다. 균주 HL278#2의 완전한 게놈 서열을 분석하였으며, 원형 염색체는 4,304,603 bp이고 57.21% G+C이고 플라스미드는 244,163 bp이고 53.14% G+C였다. 4,180개의 단백질 코딩서열이 과 49개의 tRNA와 18개의 rRNA 유전자와 함께 확인되었다. 균주 HL2708# 2의 게놈은 알콜, 말토덱스트린/전분 및 단당류 대사 유전자를 보유하고 있었다. 호염성 및 중금속 저항성을 담당하는 유전자와 방향족 및 알칸 계열 탄화수소를 대사하는 유전자를 가지고 있는 것으로 분석되었다. *Marinobacter salarius*가 질산염 및 아질산염 환원능력이 없다고 알려져 있는 것과 달리, HL2708#2 균주는 질산염/아질산염 환원 효소, 질산염/질산염 운반체 및 질산염 모노 옥시게나제를 가지고 있는 것으로 보아 세포 체외의 니트로알켄을 활용할 수 있는 능력을 가진 것으로 사료된다.

Conflict of Interest

The authors declare no conflict of interest.

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References

Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, *et al.* 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat. Methods* **10**, 563.

Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, *et al.* 2014. Pfam: the protein families database. *Nucleic Acids Res.* **42**, D222–D230.

Gauthier MJ, Lafay B, Christen R, Fernandez L, Acquaviva M, Bonin P, and Bertrand JC. 1992. *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. *Int. J. Syst. Evol. Microbiol.* **42**, 568–576.

Griffiths-Jones S, Moxon S, Marshall M, Khanna A, Eddy SR, and Bateman A. 2005. Rfam: annotating non-coding RNAs in complete genomes. *Nucleic Acids Res.* **33**, D121–D124.

Hyatt D, Chen GL, LoCascio PF, Land ML, Larimer FW, and Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* **11**, 119.

Koo CS, Sookyung S, Kim D, and Jang D. 2018. A novel marine-derived bacterium, and cosmetic compositions using the same. Vol. KR101918673B1, Republic of Korea.

Krogh A, Larsson B, von Heijne G, and Sonnhammer ELL. 2001.

Predicting transmembrane protein topology with a hidden markov model: application to complete genomes. *J. Mol. Biol.* **305**, 567–580.

Krzywinski MI, Schein JE, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, and Marra MA. 2009. Circos: An information aesthetic for comparative genomics. *Genome Res.* **19**, 1639–1645.

Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, and Breitling R. 2011. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res.* **39**, W339–W346.

Ng HJ, López-Pérez M, Webb HK, Gomez D, Sawabe T, Ryan J, Vyssotski M, Bizet C, Malherbe F, Mikhailov VV, *et al.* 2014. *Marinobacter salarius* sp. nov. and *Marinobacter similis* sp. nov., isolated from sea water. *PLoS One* **9**, e106514.

Petersen TN, Brunak S, von Heijne G, and Nielsen H. 2011. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat. Methods* **8**, 785.

Tatusov RL, Fedorova ND, Jackson JD, Jacobs AR, Kiryutin B, Koonin EV, Krylov DM, Mazumder R, Mekhedov SL, Nikolskaya AN, *et al.* 2003. The COG database: an updated version includes eukaryotes. *BMC Bioinformatics* **4**, 41.

Wu CH, Apweiler R, Bairoch A, Natale DA, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, *et al.* 2006. The universal protein resource (UniProt): an expanding universe of protein information. *Nucleic Acids Res.* **34**, D187–D191.