Phylogenomics of Haloarchaea: The Controversy of the Genera Natrinema-Haloterrigena

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The haloarchaeal genera Natrinema and Haloterrigena were described almost simultaneously by two different research groups and some strains studied separately were described as different species of these genera. Furthermore, the description of additional species was assigned to either Natrinema or Haloterrigena, mainly on the basis of the phylogenetic comparative analysis of single genes (16S rRNA gene and more recently rpoB’ gene), but these species were not adequately separated or assigned to the corresponding genus. Some studies suggested that the species of these two genera should be unified into a single genus, while other studies indicated that the genera should remain but some of the species should be reassigned. In this study, we have sequenced or collected the genomes of the type strains of species of Natrinema and Haloterrigena and we have carried out a comparative genomic analysis in order to clarify the controversy related to these two genera. The phylogenomic analysis based on the comparison of 525 translated single-copy orthologous genes and the Overall Genome Relatedness Indexes (i.e., AAI, POCP, ANI, and dDDH) clearly indicate that the species Haloterrigena hispanica, Haloterrigena lmicola, Haloterrigena longa, Haloterrigena mahii, Haloterrigena saccharevitans, Haloterrigena thermodolerans, and Halopiger salifodinae should be transferred to the genus Natrinema, as Natrinema hispanicum, Natrinema lmicola, Natrinema longum, Natrinema mahii, Natrinema saccharevitans, Natrinema thermodolerans, and Natrinema salifodinae, respectively. On the contrary, the species Haloterrigena turkmenica, Haloterrigena salifodinae, and Haloterrigena salina will remain as the only representative species of the genus Haloterrigena. Besides, the species Haloterrigena daqingensis should be reclassified as a member of the genus Natronorubrum, as Natronorubrum daqingense. At the species level, Haloterrigena jeotgali and Natrinema ejinorense should be considered as a later heterotypic synonyms of the species Haloterrigena (Natrinema) thermodolerans and Haloterrigena (Natrinema) longa, respectively. Synteny analysis and phenotypic features also supported those proposals.

Keywords: haloarchaea, Halobacteria, Natrinema, Haloterrigena, comparative genomic analysis, taxophylogenomic analysis
INTRODUCTION

Halobacteria are a monophyletic group of extremely halophilic archaea affiliated to the single class Halobacteria, belonging to the phylum Euryarchaeota (Oren et al., 2017). Currently, the class Halobacteria comprises three orders (i.e., Halobacteriales, Haloferracales, and Natrrialbales), six families (i.e., Halobacteriaceae, Haloarcuclaceae, Halococcaceae, Haloferracaceae, Halorubraceae, and Natrrialbaceae), 72 genera and 289 species whose names have been validly published (Parte et al., 2020), reflecting the high diversity and complex phylogenetic relationships within the halobacteria. In fact, recent pan-genome analysis and ancestral state reconstruction has brought to light the heterogeneity of this class, which possesses an open pan-genome, and the occurrence of genome expansion and horizontal gene transfer during the evolution of Halobacteria (Gaba et al., 2020).

The genera Natrinema and Haloterrigena are members of the family Natrrialbaceae. The genus Natrinema was described in October 1998 (McGenity et al., 1998), just 3 months earlier than the genus Haloterrigena (Ventosa et al., 1999). For that reason, the latter article did not include the recently described strains of Natrinema for comparative purposes since the manuscript was submitted for peer-review before the acceptance of the former. Therefore, Ventosa et al. (1999), honestly according to their results, proposed the creation of the new genus Haloterrigena with the new species Htg. turkmenica, instead of a novel species within the genus Natrinema, which would have been more advisable. Since then, several new species affiliated to both genera have been described and, nowadays, the genus Natrinema comprises eight validly published species names (Minegishi and Kamekura, 2019b) while Haloterrigena harbors 11 species (Chen et al., 2019; Minegishi and Kamekura, 2019a). In addition, other non-validated species names have been proposed, specifically, “Natrinema ajinwuensis” (Mahansaria et al., 2018) and “Natrinema thermophila” (Kim et al., 2018), as well as isolates not-yet assigned to any existent species (Natrinema sp. J7-1, Natrinema sp. J7-2, Haloterrigena sp. GSL-11, and Haloterrigena sp. SGH1) (Post and Al-Harjan, 1988; Zhang et al., 2012; Flores et al., 2020).

Several studies have pointed out the taxonomic problems arising in the genera Natrinema and Haloterrigena from the fact that molecular markers (i.e., 16S rRNA, atpB, EF-2, radA, rpoB, and secY gene sequences) and DNA–DNA hybridization data suggest an overlapping among members of both genera (Oren and Ventosa, 2002; Tindall, 2003; Wright, 2006; Enache et al., 2007; Minegishi et al., 2010; Papke et al., 2011). However, a detailed phylogenomic and comparative genomic study based on whole genome sequences has not been accomplished yet, nor was a formal proposal made to unravel the controversy between the clusters Natrinema/Haloterrigena. Moreover, the taxonomic status of the closely related genus Natronorurbum deserves special attention because 16S rRNA gene phylogenetic reconstructions suggest that the species Natronorurbum sediminis might belong to the Natrinema/Haloterrigena group, as the closest relative to Haloterrigena daqingensis (Ruiz-Romero et al., 2013). Since Natronorurbum sediminis (Gutiérrez et al., 2010) and Haloterrigena daqingensis (Wang et al., 2010) were proposed at almost the same time (only a 2-month gap), their close relationship was not noticed at that time. Additionally, the species Halopiger salifodinae seems to be properly affiliated to the genus Halopiger according to the 16S rRNA gene-based phylogeny, but complete rpoB gene sequence analysis (which has been demonstrated to be a more advantageous phylogenetic marker than the 16S rRNA gene in the class Halobacteria) (Minegishi et al., 2010), indicated its closest relationship with the Natrinema/Haloterrigena cluster (Minegishi et al., 2016).

In the post-genomic era, it is possible to take advantage of big genome databases and low-cost sequencing to infer phylogenetic relationships among prokaryotes using the core orthologous genes detected in the genomes under study in order to accurately elucidate their evolutionary history (de la Haba et al., 2019). Besides, comparative genomics and Overall Genome Related Indexes (OGRI) have been proposed as approaches to inspect the evolutionary distance among species and to delineate prokaryotic taxa at family, genus and species level (Borriss et al., 2011; Chun and Rainey, 2014; Konstantinidis et al., 2017; Ramirez-Durán et al., 2021) and current taxonomy should benefit from them.

Aimed to resolve the taxonomic issues in the cluster Natrinema/Haloterrigena and related taxa within the family Natrrialbaceae, we conducted phylogenomic and comparative genomic analyses using available dataset from public databanks. Additionally, we also obtained the whole genome sequence of a relevant type strain of this family which was missing in data banks. Several taxonomic changes are formally proposed in view of our results.

MATERIALS AND METHODS

Genome Retrieval and Sequencing

All genome sequences from type strains of species of the family Natrrialbaceae available until May 31st, 2020 in NCBI GenBank database were retrieved. Other additional genomes from reference (non-type) strains of Natrinema/Haloterrigena genera were also recovered (Table 1). Whole genome sequences were annotated following the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Haft et al., 2018) to predict protein-coding genes as well as other functional genome units, such as structural RNAs and tRNAs.

The genome sequence of the type strain of Haloterrigena longa was not available in any searched public database (NCBI GenBank, JGI Genome Portal, Global Catalog of Type Strain). Since that sequence data was quite relevant for the present work, we obtained the type material from the Japanese Collection of Microorganisms for the aforementioned strain (JCM 13563) and further processed it in order to obtain its whole genome sequence. High-quality genomic DNA was extracted using the QI Amp DNA Mini Kit (Qiagen) following the manufacturer’s instructions. Library preparation was performed using a combination of paired-end and mate pair strategies to generate short-insert and long-insert paired-end DNA libraries, respectively. DNA fragments were sequenced on an Illumina MiSeq platform to obtain 2 × 301-bp short-insert paired-end
| Strain                        | Accession no.                  | Assembly | Level       | Size (Mb) | GC%   | Scaffolds | Contigs | CDS | N50 | L50 |
|------------------------------|--------------------------------|----------|-------------|-----------|-------|-----------|---------|-----|-----|-----|
| Halobiforma haloterrestris   | DSM 13078                      | FOKW00000000.1 | Scaffold    | 4.50      | 65.4  | 31        | 32      | 4273| 375,716 | 4   |
| Halobiforma lacticati A15    |                                | CP019285.1 | Complete    | 4.38      | 65.2  | 3         | 3       | 4177| 4,161,587 | 1   |
| Halobiforma nitratireducens  | JCM 10879                      | AOM00000000.1 | Scaffold    | 3.69      | 63.7  | 205       | 205     | 3552| 47,406  | 25  |
| Halopiger aswanensis         | DSM 13151                      | RAP00000000.1 | Scaffold    | 4.87      | 64.4  | 17        | 18      | 4589| 1,426,401 | 2   |
| Halopiger djeltimassiliensis | IH3                            | CBM00000000.1 | Scaffold    | 3.78      | 64.2  | 6         | 55      | 3671| 1,082,527 | 2   |
| Halopiger goteimassiliensis  | IH3                            | CBM00000000.1 | Scaffold    | 3.91      | 66.1  | 3         | 11      | 3756| 3,025,424 | 1   |
| Halopiger saltolidae         | CGMCC 1.12284                  | FOIS00000000.1 | Scaffold    | 4.27      | 65.4  | 8         | 9       | 4010| 878,349  | 3   |
| Halopiger xanaduensis        | SH-6                           | NC_015666.1 | Complete    | 4.36      | 65.2  | 4         | 4       | 4178| 3,668,009 | 1   |
| Halostagnicola kamekurae     | DSM 22427                      | FOZS00000000.1 | Contig    | 4.11      | 61.5  | 16        | 16      | 4042| 2,789,326 | 1   |
| Halostagnicola larseni       | XH-48                          | CP007055.1 | Complete    | 4.13      | 60.9  | 5         | 5       | 3966| 2,789,326 | 1   |
| Haloterrigena daqingensis    | CGMCC 1.8909                   | FTNP00000000.1 | Contig    | 3.83      | 61.4  | 14        | 14      | 3687| 859,600  | 2   |
| Haloterrigena daqingensis    | JX313                          | CP019327.1 | Complete    | 3.84      | 61.3  | 4         | 4       | 3692| 3,979,437 | 1   |
| Haloterrigena hispanica      | CDM_1 FMZP00000000.1           | FMZP00000000.1 | Contig    | 3.91      | 61.0  | 135       | 139     | 3983| 148,801  | 9   |
| Haloterrigena hispanica      | CDM_6 FOIC00000000.1           | FOIC00000000.1 | Contig    | 3.96      | 61.0  | 92        | 100     | 3989| 126,565  | 9   |
| Haloterrigena hispanica      | DSM 18328                      | SHMP00000000.1 | Contig    | 4.26      | 60.7  | 11        | 11      | 4121| 1,073,359 | 2   |
| Haloterrigena jeotgali       | A29                            | CP031303.1 (chromosome), CP031298.1, CP031299.1, CP031300.1, CP031301.1, CP031302.1, CP031304.1 (plasmids) | Complete | 4.90      | 65.0  | 7         | 7       | 4967| 3,644,881 | 1   |
| Haloterrigena limicola       | JCM 13563                      | AOIT00000000.1 | Contig    | 3.52      | 61.8  | 94        | 94      | 3512| 116,493  | 9   |
| Haloterrigena longa          | JCM 13563                      | JAHUQE00000000.1 | Contig    | 4.13      | 60.9  | 6         | 6       | 4069| 3,590,587 | 1   |
| Haloterrigena mahi           | H13                            | JHUT00000000.2 | Scaffold    | 3.79      | 65.1  | 24        | 29      | 3707| 248,588  | 4   |
| Haloterrigena saccharevitans| AB14                           | LWLN00000000.1 | Scaffold    | 3.80      | 65.3  | 3         | 3       | 3921| 3,473,758 | 1   |
| Haloterrigena saltolidae     | ZY19                           | ROWN00000000.1 | Scaffold    | 4.96      | 64.5  | 11        | 14      | 4761| 1,204,032 | 2   |
| Haloterrigena salina         | JCM 13891                      | AOIS00000000.1 | Contig    | 4.84      | 65.2  | 71        | 71      | 4540| 151,334  | 11  |
| Haloterrigena sp. H1         |                                | SMZK00000000.1 | Contig    | 4.26      | 61.5  | 9         | 9       | 4253| 3,035,199 | 1   |
| Haloterrigena thermotolerans| DSM 11552                      | ADIR00000000.1 | Contig    | 3.90      | 65.4  | 68        | 68      | 3862| 162,183  | 9   |
| Haloterrigena turkmenica     | DSM 5511                       | NC_013743.1 | Complete    | 5.44      | 64.2  | 7         | 7       | 5167| 3,889,038 | 1   |
| Haloterrigena turkmenica     | WANU15 LKCV00000000.1          | ROQW00000000.1 | Contig    | 3.42      | 64.5  | 24        | 24      | 3115| 327,817  | 4   |
| Haloterrigena asiaticus      | JCM 14624                      | AOIS00000000.1 | Contig    | 3.90      | 65.4  | 68        | 68      | 3862| 162,183  | 9   |
| Haloterrigena ruber           | XH-70T                         | NC_019661.1 | Complete    | 3.23      | 64.3  | 1         | 1       | 3099| 3,223,876 | 1   |
| Natrarchaeobaculum aegepliacum| JW/NN/HA 15T                   | CP019893.1 | Complete    | 3.93      | 64.1  | 1         | 1       | 3745| 3,930,546 | 1   |
| Natrarchaeobaculum sulfurireducens | AArcht4T            | CP024047.1 | Complete    | 3.79      | 62.4  | 3         | 3       | 3576| 3,521,804 | 1   |
| Natrarchaeobius chitinivorans| AArcht4T                      | REGQ00000000.1 | Contig    | 4.57      | 61.9  | 48        | 48      | 4382| 170,161  | 8   |
| Natrarchaeobius halalkaliphilus| AArcht-StT           | REFY00000000.1 | Contig    | 3.51      | 61.1  | 12        | 12      | 3409| 639,802  | 3   |

(Continued)
| Strain                      | Accession no.          | Assembly                  | Level     | Size (Mb) | GC%  | Scaffolds | Contigs | CDS   | N50  | L50  |
|----------------------------|------------------------|---------------------------|-----------|-----------|------|-----------|---------|-------|------|------|
| Natronobacterium gregoryi SP²¹  | NC_019792.1            | Complete                  | 3.79      | 62.2      | 1    | 1         | 3710    | 3,788,356 | 1   |      |
| Natronobacterium texococonense DSM 2476T⁴ | PNLC00000000.1   | Scaffold                  | 4.01      | 62.9      | 9    | 10        | 3976    | 1,245,734 | 2   |      |
| Natronococcus amyoxylicus DSM 1052T⁴ | AOIB00000000.1           | Complete                  | 3.42      | 64.4      | 44   | 44        | 4320    | 232,276    | 7   |      |
| Natronococcus jeotgal DSM 1879G⁵  | AOIA00000000.1           | Complete                  | 4.50      | 64.4      | 170  | 170       | 4458    | 76,066     | 20  |      |
| Natronococcus occultus SP⁴¹  | NC_019974.1            | Complete                  | 3.31      | 64.6      | 3    | 3         | 4174    | 4,013,216 | 1   |      |
| Natronimidobius baerhuenensis CGMC 1.3597T  | MWPH00000000.1          | Complete                  | 3.91      | 60.2      | 8    | 8         | 3745    | 1,261,254 | 2   |      |
| Natronimidobitanis innermongolicus JCM 1225S⁵ | AOHZ00000000.1          | Complete                  | 4.59      | 64.3      | 121  | 121       | 4384    | 96,333     | 18  |      |
| Natronorubrum abyense 7-3¹  | CP045488.1             | Complete                  | 4.35      | 61.5      | 4    | 4         | 4130    | 3,352,994 | 1   |      |
| Natronorubrum bangense JCM 10635T  | AOYH00000000.1           | Complete                  | 4.11      | 60.4      | 62   | 62        | 3982    | 138,654    | 10  |      |
| Natronorubrum sedimins CGMC 1.8981T  | FNWL00000000.1           | Scaffold                  | 3.78      | 61.1      | 9    | 9         | 3583    | 1,300,740  | 2   |      |
| Natronorubrum sulfidifaciens JCM 14089T | AOCH00000000.1           | Scaffold                  | 3.46      | 61.8      | 63   | 63        | 3408    | 225,522    | 5   |      |
| Natronorubrum texococonense B⁴¹  | FNFE00000000.1           | Complete                  | 4.64      | 63.6      | 11   | 11        | 4423    | 457,630    | 3   |      |
| *Natronorubrum thiooxidans* HArc  | FTRN00000000.1           | Scaffold                  | 4.21      | 60.9      | 62   | 63        | 4067    | 250,216    | 6   |      |
| Natronorubrum tibetense GA33T | ARPH00000000.1           | Complete                  | 4.93      | 62.3      | 5    | 10        | 4649    | 4,057,512 | 1   |      |

TABLE 1 (Continued)
Nucleotide Identity (OrthoANI) was determined using the for all-vs.-all genome pairs. Specifically, Orthologous Average Overall Genome Relatedness Indexes (OGRI) were calculated Comparative Genomic Analyses v.5.7 (Letunic and Bork, 2021). 

Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999). reconstruction. Tree branch support was inferred using the rate for each site (JTT + model of amino acid evolution (Jones et al., 1992) with a single the approximately maximum-likelihood algorithm implemented further analyzed to generate the phylogenomic tree by means of and concatenated into a super-protein alignment, which was annotated genomes under study, as previously described (de la Haba et al., 2019). Then, translated single-copy core gene datasets were determined using an all-vs.-all Blastp comparison among the translated CDS features of the and core-genome datasets were determined using an all-vs.-all accurately phylogenetic approaches have been demonstrated not to be reliable to determine in-depth evolutionary relationships within the class Halobacteria and their results must be regarded with caution and carefully checked (Papke, 2009; Corral et al., 2018; de la Haba et al., 2018; Infante-Dominguez et al., 2020), a more robust and accurate phylogenetic approach was attempted. Firstly, pan- and core-genome datasets were determined using an all-vs.-all Blastp comparison among the translated CDS features of the annotated genomes under study, as previously described (de la Haba et al., 2019). Then, translated single-copy core gene sequences were individually aligned with Muscle (Edgar, 2004) and concatenated into a super-protein alignment, which was further analyzed to generate the phylogenomic tree by means of the approximately maximum-likelihood algorithm implemented in FastTreeMP v.2.1.8 (Price et al., 2010). Jones-Taylor-Thornton model of amino acid evolution (Jones et al., 1992) with a single rate for each site (JTT + CAT) was applied for phylogenomic reconstruction. Tree branch support was inferred using the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999).

Both, 16S rRNA gene-based and phylogenomic trees, were managed, displayed and annotated using the online tool iTOL v.5.7 (Letunic and Bork, 2021).

**Phylogenetic and Phylogenomic Treeing**

The 16S rRNA gene sequences from the type strains of species of the family Natrionalbaceae were downloaded from GenBank/EMBL/DDBJ databases or extracted from the whole genome sequences and then, aligned and used to calculate similarity matrixes and to construct neighbor-joining (NJ) (Saitou and Nei, 1987), maximum-parsimony (MP) (Fitch, 1971), and maximum-likelihood (MP) (Felsenstein, 1981) phylogenetic trees in ARB v.6.0.5 software package (Westram et al., 2011). Jukes-Cantor model of DNA evolution (Jukes and Cantor, 1969) was selected to correct the distance matrix. General Time Reversible model (Tavaré, 1986) with gamma-distribution and proportion of invariant sites to estimate rate heterogeneity over sites (GTR + I) was used to infer ML phylogeny. Branch support was assessed by 1,000 bootstrap pseudo-replicates (Felsenstein, 1985). Since 16S rRNA gene-based phylogenies have been demonstrated not to be reliable to determine in-depth evolutionary relationships within the class Halobacteria and their results must be regarded with caution and carefully checked (Papke, 2009; Corral et al., 2018; de la Haba et al., 2018; Infante-Dominguez et al., 2020), a more robust and accurate phylogenetic approach was attempted. Firstly, pan- and core-genome datasets were determined using an all-vs.-all Blastp comparison among the translated CDS features of the annotated genomes under study, as previously described (de la Haba et al., 2019). Then, translated single-copy core gene sequences were individually aligned with Muscle (Edgar, 2004) and concatenated into a super-protein alignment, which was further analyzed to generate the phylogenomic tree by means of the approximately maximum-likelihood algorithm implemented in FastTreeMP v.2.1.8 (Price et al., 2010). Jones-Taylor-Thornton model of amino acid evolution (Jones et al., 1992) with a single rate for each site (JTT + CAT) was applied for phylogenomic reconstruction. Tree branch support was inferred using the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999).

Both, 16S rRNA gene-based and phylogenomic trees, were managed, displayed and annotated using the online tool iTOL v.5.7 (Letunic and Bork, 2021).

**Comparative Genomic Analyses**

Overall Genome Relatedness Indexes (OGRI) were calculated for all-vs.-all genome pairs. Specifically, Orthologous Average Nucleotide Identity (OrthoANI) was determined using the OrthoANIu Tool (Yoon et al., 2017) which depends on USEARCH v8.1.1861, the digital DNA-DNA hybridization (dDDH) was inferred by means of the Genome-to-Genome Distance Calculator (GGDC) (formula 2) (Meier-Kolthoff et al., 2013), the Average Amino-acid Identity (AAI) was estimated using the aai.rb script from the Enveomics collection (Rodriguez-R and Konstantinidis, 2016) and, finally, the Percentage Of Conserved Proteins (POCP) was calculated with a homemade Perl script as described elsewhere (Qin et al., 2014).

Synteny analysis among selected representative genomes within the family Natronibales was carried out to detect conservation of homologous genes and gene order across closely related relatives. Because synteny can be affected by sequence fragmentation (Liu et al., 2018), draft genome contigs were reordered prior to infer synteny blocks using a gold standard genome (i.e., complete genome sequence) of a closely related species as a reference, using the Mauve Contig Mover functionality (Rissman et al., 2009). Conserved blocks were identified after Blastn pairwise comparisons (e-value ≤ 10^-3) between the rearranged genomes and synteny plots were visualized using Easyfig v.2.2.3 (Sullivan et al., 2011).

**RESULTS AND DISCUSSION**

**The 16S rRNA Gene Sequence Analysis Unveils the Taxonomic Problems Arising Within the Genera Haloterrigena and Natrinema**

To gain a general overview of the current taxonomic situation of the family Natrionalbaceae we reconstructed a phylogeny based on the 16S rRNA gene sequences (the most widely used molecular marker in modern prokaryotic systematics) including all type strains of the species with validly published names within that family (Figure 1). As hinted at previous studies (Tindall, 2003; Wright, 2006; Gupta et al., 2016), our results confirm that neither the genus Natrinema nor the genus Haloterrigena constituted monophyletic groups, but the constituent species of both genera were intermingled into a single monophyletic cluster, with the exception of the species Haloterrigena daqingensis which clustered together to Natronorubrum sediminis and Natronococcus roseus, distantly related to the rest of the species of Natrinema/Haloterrigena. Other problematic (polyphyletic or paraphyletic) genera within this family were Halovivax, Natrialba, Natronococcus, and Natronorubrum (Figure 1).

The 16S rRNA gene sequence similarities among the type species within the genera Natrinema and Haloterrigena, independently considered, ranged between 99.5–95.3% and 99.0–94.4%, respectively, while the sequence similarities between both genera varied from 99.0 to 94.6%, by far above the threshold value for differentiating prokaryotic genera (<94.5%) (Yarza et al., 2014). Therefore, intra- and inter-genera sequence similarities overlap almost entirely, which indicates a rather fuzzy delineation between those two genera. With regards to species delineation, the following monophyletic groups sharing equal or more than 98.65%
sequence similarity (generally accepted as the prokaryotic species cutoff value) (Kim et al., 2014) could be observed: Natrinema pellirubrum—Natrinema pallidum; Natrinema ejinorense—Haloterrigena longa; Haloterrigena mahii—Haloterrigena saccharevitans—Haloterrigena thermotolerans; Haloterrigena hispanica—Haloterrigena limicola; Haloterrigena daqingensis— Natronococcus roseus—Natronorubrum sediminis. Besides, other potential species synonymy could be detected in the family
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**Natrialbaceae: Halobiforma haloterrestris—Halobiforma lacisali; Halopiger aswanensis—Halopiger thermotolerans—Halopiger xanaduensis; Halostagnicola alkalphila—Halostagnicola bangensis; Halovivax asaticus—Halovivax ruber; and Natrialba aegyptia—Natrialba taiwanensis—Natrialba asiatica.** Despite that different species could sometimes share values above the indicated threshold, the groups mentioned here should be carefully checked to detect the existence of synonymy.

**Taxophylogenomics and Overall Genome Related Indexes Values Prove the Proposal to Keep the Genera Natrinema and Haloterrigena as Separated Taxa**

To confirm the results noted after 16S rRNA gene sequence analysis, a more robust and determinative phylogenomic analysis was carried out. For that purpose, all genome sequences from type strains of the species of the family Natrialbaceae as well as other non-type strains of the genera Natrinema and Haloterrigena available in NCBI GenBank database at the time of the study were recovered. Since the genome data for the type strain of Haloterrigena longa could not be retrieved and because this species requested special attention given its close relationship to Natrinema ejinorense (as indicated above), we sequenced and analyzed it. A total of ~0.32 and ~2.12 Gb from paired-end and mate pair libraries, respectively, were obtained after trimming and filtering. Average insert size was computationally estimated to be ~550 bp for paired-end and ~2,000 bp for mate pair datasets. Assembly yielded a 4.13 Mb, 6 scaffolds genome with a N50 of 3,590,587 bp and a coverage of 78X. **Table 1** shows all the genome sequences used in this study, as well as their main features.

Phylogenomic trees inferred from the concatenation of the 525 amino acid sequences of the orthologous single-copy genes present in the type strain genomes (**Figure 2**) and in all the genomes under study (**Supplementary Figure 1**) were obtained. A previous study focused on the evolution of the class Halobacteria has also reported phylogenomic core trees that concurs with our results, although those phylogenetic reconstructions were based on only 45 orthologous core genes and did not include all the representative genomes within the Natrialbales (Gaba et al., 2020). As might be expected, the topology of our phylogenomic tree was not totally in agreement to the 16S rRNA gene phylogeny, but the clusters obtained were better supported in the phylogenomic tree, with 100% bootstrap in almost all bifurcations. Most significantly, the strains from the cluster Natrinema/Haloterrigena (which could not be distinguished either from each other, as in the 16S rRNA tree) did not form a monophyletic group, even when excluding the species Haloterrigena daqingensis. In particular, Haloterrigena turkmenica (the type species of the genus), Haloterrigena salifodinae, and Haloterrigena salina clustered together and separated from the other Natrinema/Haloterrigena members. Therefore, our results indicate that merging both genera is not convenient, while transferring some current Haloterrigena species (i.e., Htg. hispanica, Htg. jeotgali, Htg. limicola, Htg. longa, Htg. mahii, Htg. sacharevitans, Htg. thermotolerans) to the genus Natrinema seems more appropriate. That way the genus Haloterrigena would remain composed of the species Htg. turkmenica, Htg. salina, and Htg. salifodinae. Furthermore, the species Haloterrigena daqingensis formed a monophyletic group with all the Natronorubrum species, thus suggesting its reclassification as a member of the latter genus. It is worth noting that the species Halopiger salifodinae did not affiliate with the other Halopiger species but was closely related to the Natrinema/Haloterrigena group. This taxon might belong, indeed, to the latter group or, alternatively, it might constitute a new separate genus within the Natrialbaceae. In order to unravel this issue, an in-depth analysis of OGRI values may be determinative.

The reference non-type strains whose genome sequences were included in this study showed that all of them clustered together to their respective type strain, except for the strain Haloterrigena turkmenica WANU15, which might be part of the genus Natronolimnophiles; however, it must be noted that the genome sequence of Haloterrigena turkmenica WANU15 has been confirmed to be contaminated (Lee et al., 2017) and, thus, this result must be observed with caution. Concerning the unnamed strains analyzed (i.e., Natrinema sp. J-1, Natrinema sp. J-2, and Haloterrigena sp. H1), the two first probably belong to the species Natrinema gar, whereas the latter might be regarded as a new species into the Natrinema/Haloterrigena archaeal set. Phylogenomic tree also uncover some other taxonomic problems arising within the Natrialbaceae, such as the polyphyly of the genera Natrila and Halopiger, but they are out of the scope of this study.

Aimed to shed light on the classification of the Natrialbaceae, several OGRI types were calculated, in particular those mostly accepted to delineate taxa at the prokaryotic genus and species level. Methods to demarcate genera have been proposed that are based on either AAI (Konstantinidis and Tiedje, 2007) or the POCP (Qin et al., 2014). The former approach sets a cutoff value for genus demarcation of 65% AAI (Konstantinidis et al., 2017); however, this threshold cannot be universally employed for all bacterial and archaeal lineages. In fact, if we would use the 65% AAI cutoff all the genera within the Natrialbaceae, apart from the genus Halovivax, should be merged in a single one since they shared AAI values equal or above 67% (**Figure 3** and **Supplementary Figure 2**). Therefore, AAI values might be useful for genera demarcation in this family, but a different boundary needs to be established for it. Previous studies have pointed out the convenience to set lineage specific OGRI limits to define prokaryotic genera (Barco et al., 2020). Genus demarcation boundaries were determined for the family Natrialbaceae after detailed inspection of AAI values for all pairwise genome comparisons (**Figure 3** and **Supplementary Figure 2**), in agreement with the phylogenetic trees (**Figure 2** and **Supplementary Figure 1**), to avoid the existence of polyphyletic genera. Thus, we propose a cutoff value of ≤ 76% AAI to differentiate genera within the family Natrialbaceae, a robust and consistent threshold according to the observed evolutionary relationships among members of this family. By using this threshold, the species Haloterrigena turkmenica,
FIGURE 2 | Approximate maximum-likelihood phylogenomic tree based on the concatenation of the translated sequence of the 525 single-copy genes shared by the type strains of members of the genera *Natrinema* and *Haloterrigena* and related taxa of the family *Natrialbaceae* under study. Bootstrap values $\geq 70\%$ (based on Shimodaira-Hasegawa-like local support) are shown above the branches. Bar, 0.1 changes per nucleotide position. Empty symbols indicate the type species of the corresponding genus.
Haloterrigena salifodiniae, and Haloterrigena salina will be retained as the only members of Haloterrigena. Moreover, the species Haloterrigena daqingensis should be transferred to the genus Natronorubrum. Finally, the remaining species of Haloterrigena, the species Halopiger salifodiniae and all the Natrinema species should be joined together into a single genus. Since the genus Natrinema has priority over the other two, all the aforementioned species should be reclassified as members of Natrinema. Our proposed genus limit should also have consequences in the taxonomic status of other genera of the family Natrialbaceae, such as the convenience to merge the genera Halobiforma and Natronobacterium, the transfer of Natrialba swarupia into the genus Natrarchaeobius and the need to revisit the affiliation of Halopiger goleimassiliensis and Halopiger djelfimassiliensis outside the genus Halopiger. Nevertheless, additional studies including all the type strains of the species of those genera is required, which is beyond the subject of the present article.
On the other hand, the POCP method sets a genus boundary at a value of 50% (Qin et al., 2014). Nevertheless, that limit cannot be applied to the family Natrialbaceae since all the constituent genera shared values above it. It has been discussed that this cutoff value was arbitrarily established (Barco et al., 2020), so, according to our results (Supplementary Figure 3) we can propose a threshold at a POCP value of < 66% for genus demarcation in this family. Nevertheless, this genomic index seems not to be as accurate as AAI and in borderline cases interpretation of results may be unclear. For example, the group formed by Haloterrigena turkmenica, Haloterrigena salifodinae, and Haloterrigena salina (which seemed to constitute an independent genus as explained above) could not be clearly separated from the Haloterrigena daqingensis/Natronorubrum spp. cluster, from the genus Natronolimnohabitans, or from the rest of the strains of the Natrinema/Haloterrigena clade using our proposed POCP-based genus cutoff. Another outlier was the low POCP values of the strain Natrinema altunense 1A4-DGR with respect to most of the strains within the Natrinema/Haloterrigena cluster, indicating some confidence issues for this index. Besides, other genera within the family Natrialbaceae that could not be distinguished using POCP index but whose unification is not supported by phylogenomic tree were Natrarchaeobaculum—Natrarchaeobius—Natronolimnobius; Halobiorma—Halopiger. Hence, we discourage taxonomist from using POCP method to define genera within the family Natrialbaceae.

A longer list of OGRI has been proposed to be useful for prokaryotic species delineation (Palmer et al., 2020), such as AAI (Konstantinidis and Tiedje, 2005) —which can also be employed for genus demarcation—, ANlb (Goris et al., 2007), ANIm, TETRA (Richter and Rossello-Mora, 2009), MUMi (Delger et al., 2009), dDDH (Meier-Kolthoff et al., 2013), gANI, alignment fraction (Varghese et al., 2015), OrthoANI (Lee et al., 2016), and FastANI (Jain et al., 2018). Among them, two of the most widely used for taxonomic purposes at species level are dDDH and OrthoANI, with widely accepted cutoff values of 70% (Auch et al., 2010) and 95–96% (Goris et al., 2007; Richter and Rossello-Mora, 2009; Chun and Rainey, 2014), respectively. We calculated these two OGRI for the family Natrialbaceae (Figure 4 and Supplementary Figure 4) with the aim to identify the existence of synonymy between recognized species names and to properly affiliate unnamed strains to a species. A first glimpse of OrthoANI/dDDH results showed several borderline genome pairs (94% OrthoANI and ~55% dDDH) in our dataset, in particular Haloterrigena hispanica DSM 18328T/Haloterrigena limicola JCM 13563T, Haloterrigena daqingensis JX313T/GCMM 1.8909T/Natronorubrum sediminis CGMCC 1.8961T, and Haloterrigena salifodinae ZY19T/Haloterrigena salina JCM 13891T, but they cannot be regarded as synonyms because they are still below the species threshold values and might indicate a recent speciation event. Following this criterion, the non-type strains Haloterrigena hispanica CDM_1 and Haloterrigena hispanica CDM_6 seemed to be misclassified and they should be described as a separated species from Haloterrigena hispanica, although a further descriptive characterization is required for this purpose. Unfortunately, none of both strains are available in public microbial culture collections. Similarly, the strain Haloterrigena sp. H1, sharing ≤ 89% OrthoANI and ≤ 39% dDDH values with respect to any of the analyzed strains in the family Natrialbaceae, constitutes a novel species within the cluster Natrinema/Haloterrigena, but access to the biological resource is needed before to make any formal proposal. Our study also indicated that the species “Natrinema thermophila” (Kim et al., 2018) and “Natronorubrum thiooxidans” (Sorokin et al., 2005) (names effectively but not validly published) should be unequivocally considered as novel taxa within their respective genera, although those names need to be validated beforehand. More uncertain was the taxonomic differentiation of several genome pairs within the fuzzy zone (95% OrthoANI and 60–63% dDDH), specifically Natrinema pellirubrum DSM 15624T/Haloterrigena jeotgali A29T, Natrinema pellirubrum DSM 15624T/Haloterrigena thermotolerans DSM 11522T, and Natrinema ejiinorenc JCM 13890T/Haloterrigena longa JCM 13563T. Additionally, it must be noted that when using formula 1 and 3 (instead of formula 2) for dDDH calculation the results for the aforementioned genome pairs were 64–65%, 67–68%, and 70%, respectively, making more challenging their proper taxonomic classification. In those cases, the sole use of OGRI values was not discriminative enough as to make a decision on their taxonomy and additional genomic and phenotypic data must be provided. On the other hand, OrthoANI and dDDH values doubtlessly indicate that each of the following groups of strains belongs to the same species: Natrinema gari JCM 14663T/Natrinema sp. J7-1/Natrinema sp. J7-2, Natrinema pallidum DSM 3751T/Natrinema pallidum BOL6-1, Natrinema altunense JCM 12890T/Natrinema altunense A2T/Natrinema altunense 4.1R/Natrinema altunense 1A4-DGR, Haloterrigena hispanica CDM_1/Haloterrigena hispanica CDM_6, Haloterrigena jeotgali A29T/Haloterrigena thermotolerans DSM 11522T, Haloterrigena daqingensis JX313T/Haloterrigena daqingensis CGMCC 1.8909T, and Natronolimnohabitans innermongolicus JCM 12255T/Haloterrigena turkmenica WANU15. Therefore, the species Haloterrigena jeotgali should be considered as a later heterotypic synonym of Haloterrigena thermotolerans and the strains Natrinema sp. J7-1, Natrinema sp. J7-2, and Haloterrigena turkmenica WANU15 should be renamed as Natrinema gari J7-1, Natrinema gari J7-2, and Natronolimnohabitans innermongolicus WANU15, respectively. Other putative ambiguous synonyms were detected, such as those for the species Natrailba aegeyptia/Natrailba taiwanensis and Halobiorma haloterrestris/Halobiorma lacisali, but the convenience to be merged or not should be accomplished in future studies.

Synteny Analysis Applied to Elucidation of Uncertain Synonyms Into the Natrinema/Haloterrigena

The evolutionary processes that lead to diversity, chromosomal dynamics, and rearrangement rates between species can be assessed by means of the analysis of the synteny among two or more genomes, that is, the spatial distribution of locally collinear
blocks (Bhutkar et al., 2006). Thus, an approach to gain insight into the evolutionary distance between two species is to inspect the synteny of the genome sequences under study (Borriss et al., 2011; Ramírez-Durán et al., 2021). As indicated in the previous section, OGRl values equal to the species cutoffs were not able to reliably solve the taxonomic status of several species and so, the synteny analysis might shed light to elucidate the affiliation of those uncertain taxa.

Specifically, we have evaluated, on the one hand, the synteny between *Natrinema ejinorense* JCM 13890T and *Haloterrigena longa* JCM 13563T and, on the other hand, the synteny among *Natrinema pellirubrum* DSM 15624T, *Haloterrigena jeotgali* A29T, and *Haloterrigena thermotolerans* DSM 11523T (Figure 5). As can be observed, although some genomic rearrangements could be evidenced, all comparisons showed high levels of conservation of locally collinear blocks. It
must be noted that the synteny between Haloterrigena jeotgali A29 and Haloterrigena thermotolerans DSM 11522 seemed to be more disorganized than that for other genome pairs; however, this fact is due to the elevated fragmentation of the genome sequence from Haloterrigena thermotolerans DSM 11522 (68 scaffolds and a N50 of 162,183 bp), which reduces the robustness of the synteny analysis. In any case, the synteny results are not so relevant for such genome pair since OGRI values undoubtedly demonstrated the synonym between those species, as stated earlier. The other genome sequences analyzed here for synteny comparisons possessed high-quality, with a minimum N50 of 3.59 Mb and, therefore, they met the requirements to be confidently used for this purpose (Liu et al., 2018).

Our results concerning the study of regions of local collinearity support the union of Natrinema ejinorense and Haloterrigena longa and of Natrinema pellirubrum and Haloterrigena jeotgali/Haloterrigena thermotolerans as a single species, respectively. Nevertheless, phenotypic features should also be considered before those proposals can be formulated.

**Phenotypic Characteristics Endorse the Taxonomic Rearrangements for the Genera Natrinema and Haloterrigena**

For an accurate classification of a taxon, three major premises should be fulfilled: (i) monophyly, (ii) genomic coherence, and (iii) phenotypic coherence (Rosselló-Móra and Amann, 2015). In the previous sections we have examined the two first criterias (phylogenetic/phylogenomic trees and OGRI/synteny), but any formal taxonomic proposal should also be supported by phenotypic characters.

The species Haloterrigena turkmenica, Haloterrigena salifodinae, and Haloterrigena salina, which we propose to be retained as members of the genus Haloterrigena, shared a bunch of characteristics (Table 2), such as the coccoid morphology, the red pigmentation, the resistance to lysis in distilled water, the high salt concentration for optimal growth (>15% (w/v) NaCl), the inability to produce gas from nitrate, to form indole and H2S, and to hydrolyze starch, gelatin and Tween 80, the ability to use D-glucose, D-mannose and lactose as a sole carbon and energy sources, the presence of phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me) and mannose-2,6-disulfate (1→2)-glucose glycerol diether (S2-DGD-1) as membrane polar lipids, and the lack of phosphatidylglycerol sulfate (PGS). On the other hand, the species Haloterrigena daqingensis, which formed a monophyletic cluster with the species of the genus Natronorubrum, showed some phenotypic similarities with the species of the latter genus, remarkably, the haloalkaliphilic behavior, the inability to hydrolyze casein and to assimilate D-ribose, D-mannitol and sorbitol, the presence of PG and PGP-Me, and the absence of PGS (Table 2). Finally, the remaining species of Haloterrigena together to the species of the genus Natrinema and Halopiger salifodinae lysed in distilled water, grew optimally in media with 15–29% (w/v) NaCl, utilized acetate but not D-mannitol as the only carbon and energy sources, and possessed PG and PGP-Me as major polar lipids (Table 2). Some differences in the minor polar lipid composition of this Natrinema/Haloterrigena/Halopiger salifodinae group can be observed, in particular, the presence of S2-DGD-1 glycolipid in some species and its absence in others, and the lack of PGS in several taxa but not in all of them. Although previous studies have shown that there are
TABLE 2 | Main comparative phenotypic features among members of the genera *Natrinema* (including the species *Halopiger salifodinae*), *Haloterrigena*, and *Natronorubrum*.

| Characteristics                                      | Nnm. altunense<sup>a</sup> | Nnm. ejinorense<sup>b</sup> | Nnm. gari<sup>c</sup> | Nnm. pallidum<sup>d</sup> | Nnm. pellirubrum<sup>g</sup> | Nnm. salaciae<sup>e</sup> | Nnm. soli<sup>f</sup> | Nnm. versiforme<sup>g</sup> | Htg. hispanica<sup>h</sup> |
|------------------------------------------------------|-----------------------------|----------------------------|-----------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Morphology                                           | Rods                        | Pleomorphic                 | Rods                  | Rods                        | Rods                        | Pleomorphic                 | Oval                        | Pleomorphic                 | Coccoid                     |
| Cell size                                            | 0.8–1.2 × 3.0–7.0           | 0.8–2.0 × 1.5–4.0           | 0.5–0.8 × 2.0–3.0     | 0.7–1.0 × 1.5–6.0           | 0.6–1.0 × 1.0–4.0           | 0.8–1.5 × 1.0–3.0           | 1.2–1.6                     | ND                          | 1.5–2.0                     |
| Motility                                             | +                           | –                          | +                     | +                           | +                           | –                           | –                           | –                           | –                           |
| Colony pigmentation                                  | Orange or red               | Light red                  | Pale orange           | Pale orange, beige or almost colorless | Light red or orange | Red                        | Cream                       | Light red                   | Light red                   |
| Cells lyse in distilled water                         | ND                          | ND                         | ND                    | ND                          | ND                          | ND                          | ND                          | ND                          | +                           |
| NaCl range, % (w/v) (optimum)                        | >10 (17.5–25)               | >10.5 (20)                 | >10 (15–20)          | >10 (20–25)                 | >12 (20–25)                 | 10–30 (15–20)              | 17.5–26 (23)                | >9 (20–25)                  | 13–23 (20)                  |
| MgCl<sub>2</sub> requirement                         | +                           | –                          | ND                    | –                           | ND                          | ND                          | –                           | +                           | –                           |
| Temperature range (optimum)                          | 20–60 (37–40)               | 25–50 (37)                 | 20–60 (37–40)        | 25–60 (37–40)               | 20–45 (30–37)               | 30–52.5 (45)               | 25–45 (40)                  | 20–53 (37–46)               | 37–60 (50)                  |
| pH range (optimum)                                   | 6.0–8.0 (7.0)               | 6.0–8.5 (7.0)              | 5.5–8.5              | 6.0–8.4                     | 6.0–8.6                     | 6.5–9.0                     | 6.0–8.0 (7.0)               | 6.0–8.0 (6.5–7.0)           | 6.5–8.5 (7.0)               |
| Anaerobic growth in presence of:                     |                             |                            |                       |                             |                             |                             |                             |                             |                             |
| Nitrate                                              | +                           | –                          | –                     | +                           | –                           | +                           | –                           | +                           | ND                          |
| L-arginine                                           | ND                          | –                          | ND                    | ND                          | ND                          | –                           | –                           | ND                          | ND                          |
| Oxidase                                              | +                           | +                          | –                     | +                           | –                           | +                           | –                           | +                           | +                           |
| Catalase                                             | +                           | +                          | ND                    | ND                          | ND                          | +                           | ND                          | +                           | +                           |
| Nitrate reduction to nitrite                          | +                           | +                          | –                     | +                           | +                           | –                           | +                           | +                           | ND                          |
| Gas from nitrate                                     | +                           | +                          | –                     | –                           | –                           | ND                          | ND                          | +                           | ND                          |
| Indole production                                    | –                           | –                          | –                     | –                           | –                           | ND                          | –                           | +                           | +                           |
| H<sub>2</sub>S production                             | +                           | –                          | ND                    | –                           | ND                          | –                           | ND                          | +                           | +                           |
| Hydrolysis of:                                       |                             |                            |                       |                             |                             |                             |                             |                             |                             |
| Starch                                               | –                           | +                          | –                     | –                           | +                           | –                           | –                           | ND                          | +                           |
| Casein                                               | –                           | –                          | ND                    | –                           | –                           | –                           | –                           | –                           | –                           |
| Gelatin                                              | +                           | +                          | +                     | +                           | –                           | –                           | –                           | –                           | –                           |
| Tween 80                                              | +                           | +                          | –                     | +                           | –                           | ND                          | +                           | –                           | +                           |

(Continued)
TABLE 2 | (Continued)

| Characteristics | Nnm. altunense<sup>a</sup> | Nnm. ejinorense<sup>b</sup> | Nnm. gari<sup>c</sup> | Nnm. pallidum<sup>d</sup> | Nnm. pellirubrum<sup>d</sup> | Nnm. salacie<sup>e</sup> | Nnm. soli<sup>f</sup> | Nnm. versiforme<sup>g</sup> | Htg. hispanica<sup>h</sup> |
|-----------------|--------------------------|--------------------------|----------------------|-------------------------|-------------------------|------------------------|------------------------|------------------------|------------------------|
| **Utilization as sole carbon and energy source of:** |             |             |                      |                         |                         |                        |                        |                        |                        |
| Acetate         | +            | +            | ND                   | ND                      | ND                      | +                      | ND                     | ND                     | +                      |
| D-Glucose       | +            | +            | +                    | +                       | +                       | +                      | +                      | +                      | +                      |
| D-Fructose      | −            | +            | ND                   | ND                      | −                       | −                      | ND                     | −                      | ND                     |
| D-Galactose     | −            | −            | −                    | ND                      | ND                      | ND                     | +                      | −                      | −                      |
| D-Mannose       | +            | −            | −                    | −                       | +                       | −                      | +                      | −                      | −                      |
| D-Ribose        | −            | −            | −                    | −                       | +                       | −                      | −                      | −                      | −                      |
| D-Xylose        | −            | −            | −                    | −                       | −                       | −                      | +                      | −                      | +                      |
| Sucrose         | −            | +            | −                    | ND                      | ND                      | −                       | −                      | ND                     | −                      |
| Maltose         | +            | +            | −                    | ND                      | ND                      | −                       | −                      | ND                     | −                      |
| Lactose         | −            | −            | −                    | −                       | +                       | −                      | −                      | −                      | ND                     |
| Glycerol        | +            | +            | +                    | ND                      | ND                      | −                       | ND                     | +                      | +                      |
| Sorbitol        | ND           | −            | −                    | ND                      | ND                      | −                       | −                      | ND                     | ND                     |
| D-Mannitol      | ND           | −            | −                    | ND                      | ND                      | −                       | ND                     | ND                     | ND                     |
| **Acid production from:** |             |             |                      |                         |                         |                        |                        |                        |                        |
| D-Mannose       | +            | −            | ND                   | ND                      | ND                      | +                      | −                      | ND                     | ND                     |
| D-Glucose       | +            | −            | ND                   | ND                      | ND                      | +                      | −                      | ND                     | ND                     |
| **Lipids:**     |             |             |                      |                         |                         |                        |                        |                        |                        |
| Major polar lipids | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me |
| Major glycolipids | Unidentified glycolipid | Unidentified glycolipid | Unidentified glycolipid | Unidentified glycolipid | Unidentified glycolipid | S<sub>2</sub>-DGD-1 | S<sub>2</sub>-DGD-1 | S<sub>2</sub>-DGD-1 | S-DGD-1 |
| Presence of PGS | +            | −            | +                    | +                       | +                       | +                      | −                      | +                      | −                      |

(Continued)
| Characteristics | 
|-----------------|
| **Morphology** | 
| Rods | Rods | Rods | Rods | Rods | Rods | Pleomorphic | 
| **Cell size** | 0.4 × 1.0 | 0.6–0.8 × 1.8–3.6 | 0.5–0.6 × 2.8–11.0 | 3.0–10.0 | 0.4–1.0 × 3.0–10.0 | 0.7–1.0 × 4.0–13.0 | 0.1–0.3 | 1.1–1.5 × 1.1–1.7 | 
| **Motility** | – | + | – | – | – | – | – | – | + | 
| **Colonel pigmentation** | Light red | Red | Red | Deep red | Light red | Pale red | Cream | Pale red | Light red | Red or light pink | 
| **NaCl range, % (w/v) (optimum)** | 10–30 (15–20) | 10–30 (18) | 12–30 (20–29) | >10 (17.5–20) | 12–25 (17.5–20) | 11–32 (17–20) | 10–30 (20–25) | 15–29 | >12 (15–20) | 
| **MgCl<sub>2</sub> requirement** | – | + | – | ND | – | – | – | – | + | 
| **Temperature range (optimum)** | 17–50 (37–45) | 30–61 (40–45) | 30–56 (41–45) | 35–55 (45–55) | 24–68 (42–45) | 25–60 (50) | 25–50 (37–45) | 20–55 (42) | 25–50 | 20–55 (45) | 
| **pH range (optimum)** | 6.5–8.5 (7.0–7.5) | 6.5–9.0 (7.0) | 6.5–9.0 (7.0–7.5) | 6.0–9.0 (6.5–8.2) | 6.5–8.5 (7.5) | 6.5–8.2 (7.0–7.5) | 6.0–8.0 (7.0) | 6.0–9.5 (7.5–8.0) | 6.0–9.0 | ND | 
| **Anaerobic growth in presence of:** | 
| Nitrate | + | – | – | ND | + | – | – | – | – | ND | 
| L-arginine | ND | – | – | ND | ND | – | – | – | – | ND | 
| Oxidase | – | + | + | – | + | + | + | – | + | – | 
| Catalase | + | + | + | + | + | + | + | + | ND | 
| Nitrate reduction to nitrite | – | – | – | – | – | – | – | – | + | + | 
| Gas from nitrate | ND | ND | ND | ND | – | – | ND | – | – | ND | 
| Indole production | + | – | + | + | – | – | – | – | – | – | 
| H<sub>2</sub>S production | ND | + | + | + | + | + | + | – | – | – | 

(Continued)
TABLE 2 | (Continued)

| Characteristics | Htg. jeotgal® | Htg. limicola® | Htg. longa® | Htg. mahii® | Htg. saccharovitans® | Htg. thermodetrons® | Htg. salifodinae® | Htg. salifodinae® | Htg. salina® | Htg. turkmenica® |
|------------------|---------------|---------------|-------------|-------------|---------------------|---------------------|-----------------|-----------------|--------------|---------------|
| Hydrolysis of:   |               |               |             |             |                     |                     |                 |                 |              |               |
| Starch           | –             | –             | –           | –           | –                   | –                   | –               | –               | –            | –             |
| Casein           | +             | –             | –           | –           | –                   | –                   | –               | –               | ND           | ND            |
| Gelatin          | –             | –             | –           | –           | +                   | –                   | –               | –               | –            | –             |
| Tween 80         | +             | –             | –           | +           | +                   | +                   | –               | –               | –            | –             |
| Utilization as sole carbon and energy source of: |               |               |             |             |                     |                     |                 |                 |              |               |
| Acetate          | +             | +             | +           | ND          | ND                  | +                   | +               | +               | ND           | +             |
| D-Glucose        | –             | –             | +           | +           | –                   | –                   | +               | +               | +            | +             |
| D-Fructose       | +             | –             | –           | +           | –                   | –                   | –               | +               | –            | +             |
| D-Galactose      | ND            | –             | –           | ND          | –                   | –                   | –               | –               | ND           | ND            |
| D-Mannose        | ND            | –             | –           | ND          | –                   | +                   | +               | +               | +            | +             |
| D-Ribose         | ND            | –             | –           | –           | –                   | –                   | –               | –               | –            | –             |
| D-Xylose         | ND            | –             | –           | –           | –                   | –                   | –               | –               | –            | –             |
| Sucrose          | –             | –             | +           | +           | –                   | –                   | ND              | +               | –            | +             |
| Maltose          | ND            | –             | +           | ND          | –                   | –                   | –               | –               | +            | ND            |
| Lactose          | +             | –             | –           | +           | –                   | –                   | –               | –               | +            | +             |
| Glycerol         | ND            | ND            | ND          | ND          | ND                  | +                   | –               | +               | ND           | ND            |
| Sorbitol         | ND            | –             | ND          | ND          | ND                  | –                   | ND              | –               | –            | ND            |
| D-Mannitol       | ND            | –             | ND          | –           | –                   | –                   | –               | –               | ND           | ND            |
| Acid production from: |         |               |             |             |                     |                     |                 |                 |              |               |
| D-Mannose        | ND            | –             | –           | –           | –                   | +                   | –               | –               | +            | +             |
| D-Glucose        | ND            | –             | +           | –           | –                   | –                   | –               | –               | –            | –             |
| Lipids:          |               |               |             |             |                     |                     |                 |                 |              |               |
| Major polar lipids | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me |
| Major glycolipids | S₂-DGD-1     | S₂-DGD-1     | S₂-DGD-1   | S₂-DGD-1   | S₂-DGD-1 | S₂-DGD-1 | S₂-DGD-1 | S₂-DGD-1 | S₂-DGD-1 | S₂-DGD-1 |
| Presence of PGS | –             | –             | –           | –           | –                   | –                   | –               | –               | –            | –             |

(Continued)
| Characteristics | Nrr. albiense<sup>a</sup> | Nrr. bangense<sup>b</sup> | Nrr. halophilum<sup>c</sup> | Nrr. sediminis<sup>d</sup> | Nrr. sulfidificiens<sup>e</sup> | Nrr. texcoconense<sup>f</sup> | Nrr. tibetense<sup>g</sup> | Htg. daqingensis<sup>h</sup> |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Morphology      | Rods           | Pleomorphic    | Pleomorphic    | Pleomorphic    | Pleomorphic    | Pleomorphic    | Pleomorphic    | Coccoid        |
| Cell size       | 0.8–1.0 ×      | ND             | ND             | 0.8–1.0 ×      | ND             | 0.8–1.0 ×      | ND             | 0.8–1.3        |
| Motility        | +              | –              | +              | –              | +              | –              | –              | –              |
| Colony pigmentation | Red         | Red           | Red            | Pink           | Red            | Pink-red       | Red            | Orange         |
| NaCl range, % (w/v) (optimum) | 12–25 (15–18) | 12–25 (22.5) | 8–28 (15–18) | 15–29 (20)    | 12–28 (18)    | 10–25 (15–20) | 12–30 (20)    | 10–32 (12–15) |
| MgCl<sub>2</sub> requirement | –             | ND             | –              | –              | ND             | –              | –              | ND             |
| Temperature range (optimum) | 20–50 (45)   | 25–55 (45)    | 25–42 (37)    | 25–50 (37)    | 20–55 (44–47)| 25–45 (37)    | 25–55 (45)    | 20–50 (35)    |
| pH range (optimum) | 6.5–9.5 (8.0) | 8.0–11.0 (9.5)| 5.5–9.5 (7.0–7.5) | 8.0–11.0 (9.0) | 8.0–10.0 (8.7–9.2) | 8.0–10.5 (9.0) | 8.5–11.0 (9.0) | 8.0–10.5 (10.0) |
| Anaerobic growth in presence of: | | | | | | | | |
| Nitrate         | –              | –              | –              | –              | –              | –              | –              | –              |
| L-arginine      | –              | ND             | –              | –              | –              | –              | –              | ND             |
| Oxidase         | +              | +              | +              | +              | +              | +              | +              | +              |
| Catalase        | +              | +              | +              | +              | +              | +              | +              | +              |
| Nitrate reduction to nitrite | +          | –              | +              | +              | +              | –              | –              | –              |
| Gas from nitrate | +             | –              | v              | –              | +              | –              | –              | ND             |
| Indole production | +            | +              | +              | –              | +              | –              | +              | –              |
| H<sub>2</sub>S production | –            | –              | v              | –              | +              | –              | –              | +              |
| Hydrolysis of:  | | | | | | | | |
| Starch          | –              | –              | +              | –              | –              | –              | –              | –              |
| Casein          | –              | –              | v              | –              | –              | –              | –              | –              |
| Gelatin         | –              | –              | v              | –              | –              | –              | +              | –              |
| Tween 80        | –              | –              | v              | –              | –              | –              | –              | +              |
| Utilization as sole carbon and energy source of: | | | | | | | | |
| Acetate         | ND             | +              | –              | –              | +              | ND             | +              | +              |
| D-Glucose       | +              | +              | v              | +              | +              | +              | +              | +              |
| Characteristics | \( \text{Nrr. aibiense}^a \) | \( \text{Nrr. bangense}^a \) | \( \text{Nrr. halophilum}^a \) | \( \text{Nrr. sediminis}^a \) | \( \text{Nrr. sulfidifaciens}^a \) | \( \text{Nrr. texcoconense}^a \) | \( \text{Nrr. tibetense}^a \) | \( \text{Htg. daqingensis}^a \) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| D-Fructose | – | + | – | + | – | ND | + | – |
| D-Galactose | + | – | + | – | – | ND | – | – |
| D-Mannose | – | – | – | – | – | – | – | – |
| D-Ribose | – | – | – | – | – | ND | – | – |
| D-Xylose | – | – | – | + | – | ND | – | – |
| Sucrose | – | + | – | – | – | – | – | – |
| Maltose | – | + | – | – | – | – | – | – |
| Lactose | – | – | – | – | – | ND | – | – |
| Glycerol | ND | ND | + | – | + | ND | – | + |
| Sorbitol | – | ND | – | – | – | ND | ND | – |
| D-Mannitol | – | – | – | – | – | – | – | – |
| Acid production from: | | | | | | | | |
| D-Mannose | – | ND | + | – | ND | ND | ND | – |
| D-Glucose | + | ND | + | – | ND | ND | ND | – |
| Lipids: | | | | | | | | |
| Major polar lipids: \( \text{PG}, \text{PGP-Me} \) | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me | PG, PGP-Me |
| Major glycolipids: \( S_2-\text{DGD-1}, \text{TGD-1} \) | None | None | S\(_2\)-DGD-1, TGD-1 | None | None | None | None | S\(_2\)-DGD-1 |
| Presence of PGS | – | – | – | – | – | – | – | – |

+ , positive; –, negative; ND, not determined; ±, doubtful; v, variable. Species that should be regarded as member of the genera Natrinema, Haloterrigena, or Natronorubrum are marked in light orange, light blue, and light green, respectively.

\(^a\)Data from Xu et al. (2005b).
\(^b\)Data from Castillo et al. (2006).
\(^c\)Data from Tapingkae et al. (2008).
\(^d\)Data from McGentty et al. (1998).
\(^e\)Data from Albuquerque et al. (2012).
\(^f\)Data from Rasooli et al. (2017).
\(^g\)Data from Xin et al. (2000).
\(^h\)Data from Romano et al. (2007).
\(^i\)Data from Roh et al. (2009).
\(^j\)Data from Cui et al. (2006a).
\(^k\)Data from Ding et al. (2017).
\(^l\)Data from Xu et al. (2005a).
\(^m\)Data from Montalvo-Rodríguez et al. (2000).
\(^n\)Data from Zhang et al. (2013).
\(^o\)Data from Chen et al. (2019).
\(^p\)Data from Gutiérrez et al. (2008).
\(^q\)Data from Zvyagintseva and Tarasov (1987) and Ventosa et al. (1999).
\(^r\)Data from Cui et al. (2006a).
\(^s\)Data from Xu et al. (1999).
differences in the polar lipid composition between the species of the genera Natrinema and Haloterrigena—with Natrinema species harboring PGS but not S₂-DGD-1 (McGenity et al., 1998; Xin et al., 2000; Xu et al., 2005b; Tapingkæ et al., 2008), while Haloterrigena representatives containing S₂-DGD-1 and lacking PGS (Montalvo-Rodríguez et al., 2000; Xu et al., 2005a; Cui et al., 2006b; Roh et al., 2009; Ding et al., 2017)—we observed that minor polar lipid profiles are not genus-specific. For example, Natrinema ejinorense and Natrinema soli possessed S₂-DGD-1 and lacked PGS, and Natrinema salicola contained S₂-DGD-1 (characteristic profiles of Haloterrigena species). On the contrary, Haloterrigena hispanica did not hold S₂-DGD-1 (typical profile of Natrinema species). Therefore, those differences in minor polar lipid composition cannot be regarded as phenotypic incoherence within the Natrinema/Haloterrigena/Halopiger salifodinae cluster, whose species should be merged into the single genus Natrinema.

With respect to genera differentiation, the genuine genus Haloterrigena (Haloterrigena turkmenica, Haloterrigena salifodinae, and Haloterrigena salina) can be distinguished from the now expanded genus Natrinema (Natrinema/Haloterrigena/Halopiger salifodinae group) by the resistance to cell lysis in distilled water of the former but not of the latter. Likewise, members of the genus Natronorubrum (now also including the species Haloterrigena daqingensis) are haloalkalophiles, in contrast to their Haloterrigena and Natrinema counterparts which better thrive at almost neutral pH values (Table 2).

At the species level, phenotypic features can also shed light on uncertain taxa. This is the case of the cluster Natrinema pellirubrum/Haloterrigena jeotgali/Haloterrigena thermotolerans and the cluster Natrinema ejinorense/Haloterrigena longa, for which OGRI values fell in the fuzzy zone and synteny analysis agreed with the possibility of merging the species within each cluster. A careful inspection of the phenotypic characteristics of Natrinema pellirubrum, Haloterrigena jeotgali, and Haloterrigena thermotolerans demonstrated a similar profile for the two latter, whereas the former showed significant differences as to be considered as a separated species, such as the cell motility, the absence of S₂-DGD-1 glycolipid and the presence of PGS (Table 2). On the contrary, phenotypic profile for the species Natrinema ejinorense and Haloterrigena longa was quite similar, with only minor strain-specific differences (Table 2), thus supporting the unification of both taxa into a single species.

Taxonomic Consequences

After having completed detailed phylogenomic, genomic and phenotypic comparative analyses in the family Natroidaeaceae, and more specifically in the genera Natrinema and Haloterrigena, we have demonstrated that the species Haloterrigena jeotgali and Natrinema ejinorense should be considered as later heterotypic synonyms of the species Haloterrigena thermotolerans and Haloterrigena longa, respectively, according to Rule 23a of the International Code of Nomenclature of Prokaryotes (Parker et al., 2019). Additionally, the species Haloterrigena hispanica, Haloterrigena limicola, Haloterrigena longa/Natrinema ejinorense, Haloterrigena mahii, Haloterrigena saccharovorans, Haloterrigena thermotolerans/Haloterrigena jeotgali, and Halopiger salifodinae should be transferred to the genus Natrinema, as Natrinema hispanicum, Natrinema limicola, Natrinema longum, Natrinema mahii, Natrinema saccharovorans, Natrinema thermotolerans, and Natrinema salifodinae, respectively. On the contrary, the species Haloterrigena turkmenica, Haloterrigena salifodinae, and Haloterrigena salina will remain as the only representative species of the genus Haloterrigena. Besides, the species Haloterrigena daqingensis should be reclassified as a member of the genus Natronorubrum, as Natronorubrum daqingense.

With regards to non-type or unnamed strains, our study indicates that the strains Natrinema sp. J7-1, Natrinema sp. J7-2, and Haloterrigena turkmenica WANU15 should be renamed as Natrinema gari J7-1, Natrinema gari J7-2, and Natronolimnohabitans innermongolicus WANU15, respectively, although it is worth mentioning that the genome sequence of Haloterrigena turkmenica WANU15 has been identified as contaminated in a previous study (Lee et al., 2017). Moreover, the strains Haloterrigena hispanica CDM_1 and Haloterrigena hispanica CDM_6 should not be longer affiliated to the species Haloterrigena (Natrinema) hispanica and, thus, they should be referred as Natrinema sp. CDM_1 and Natrinema sp. CDM_6, respectively.

On the basis of these data, we propose the following taxonomic re-arrangements.

**Description of Natrinema hispanicum comb. nov.**

*Natrinema hispanicum* (his.pa’ni.cum. L. neut. adj. hispanicum of Hispania, from where the organism was originally isolated)

Basonym: *Haloterrigena hispanica* Romano et al., 2007, 1501.

The description is identical to that of *Haloterrigena hispanica* as given previously (Romano et al., 2007) with the following amendments: the G + C content of the type strain genome is 60.7 mol%, its approximate size 4.26 Mb, and its GenBank Assembly accession number is GCA_004217335.1.

The type strain is FP1\(^T\) (= ATCC BAA-1310\(^T\) = DSM 18328\(^T\)).

**Description of Natrinema limicola comb. nov.**

*Natrinema limicola* (li.mi’co.la. L. masc. n. limus mud; L. suff. -cola from L. masc. or fem. n. incola dweller; N.L. n. limicola mud-dweller)

Basonym: *Haloterrigena limicola* Cui et al., 2006b, 1839.

The description is identical to that of *Haloterrigena limicola* as given previously (Cui et al., 2006b) with the following amendments: the G + C content of the type strain genome is 61.8 mol%, its approximate size 3.52 Mb, and its GenBank Assembly accession number is GCA_000337475.1.

The type strain is AX-7\(^T\) (= CGMCC 1.5332\(^T\) = JCM 13563\(^T\)).

**Description of Natrinema longum comb. nov.**

*Natrinema longum* (lon’gum. L. neut. adj. longum long, referring to the production of long rods in liquid medium)

Basonym: *Haloterrigena longa* Cui et al., 2006b, 1838.
The description is identical to that of *Haloterrigena longa* as given previously (Cui et al., 2006b) with the amendments as follows. Cells are rod-shaped or pleomorphic (0.5–2.0 × 1.5–11.0 µm). Aerobic growth occurs at pH 6.0–9.0 and 25–56°C. Optimal NaCl concentration and temperature for growth are 18–20% (w/v) and 37–45°C, respectively. Nitrate reduction to nitrite is variable. Indole and H₂S formation are variable. Hydrolysis of starch, gelatin and Tween 80 is variable. Assimilation of fructose as carbon and energy sources is variable. Acid production from glucose and sucrose is variable. Phosphatidylglycerol sulfate polar lipid is absent or below detection limit. The DNA G + C content is 61.8–63.9 mol% (genome).

The type strain is ABH32T (= CGMCC 1.5334T = JCM 13562T). The G + C content of the type strain genome is 61.8 mol%, its approximate size 3.52 Mb, and its GenBank Assembly accession number is GCA_002105915.1.

*Natrinema eijorensis* EJ-57 (= CECT 7144 = CGMCC 1.6202 = DSM 18194 = JCM 13890) is an additional strain of *Natrinema longa*. The G + C content of this reference strain genome is 63.9 mol%, its approximate size 4.48 Mb, and its GenBank Assembly accession number is GCA_002494345.1.

**Description of Natrinema mahii comb. nov.**

*Natrinema mahii* (mah’i.i. N.L. gen. n. mahii of Mah, in honor of R.A. Mah at UCLA for his noteworthy research in the areas of archaea isolation and classification, and also for initiating the solar saltern sampling in the original description)

Basonym: *Haloterrigena mahii* Ding et al., 2017, 1337.

The description is identical to that of *Haloterrigena mahii* as given previously (Ding et al., 2017) with the following amendments: the G + C content of the type strain genome is 65.1 mol%, its approximate size 3.79 Mb, and its GenBank Assembly accession number is GCA_0002494345.1.

The type strain is H13T (= BCRC 910151T = NBRC 111885T).

**Description of Natrinema saccharivores comb. nov.**

*Natrinema saccharivores* (sac.char.e.vi’tans. L. neut. n. saccharon, -i a kind of sugar; L. pres. part. evitans shunning, avoiding; N.L. part. adj. saccharivores sugar-avoiding, because it uses very few sugars)

Basonym: *Haloterrigena saccharivores* Xu et al., 2005a, 2541.

The description is identical to that of *Haloterrigena saccharivores* as given previously (Xu et al., 2005a) with the following amendments: the G + C content of the type strain genome is 65.3 mol%, its approximate size 3.98 Mb, and its GenBank Assembly accession number is GCA_000690595.2.

The type strain is AB14T (= AS 1.3730T = JCM 12889T).

**Description of Natrinema thermotolerans comb. nov.**

*Natrinema thermotolerans* (ther.mo.to’le.rans. Gr. fem. n. therme heat; L. pres. part. tolerans tolerating; N.L. part. adj. thermotolerans heat-tolerant)

Basonym: *Haloterrigena thermotolerans* Montalvo-Rodriguez et al., 2000, 1070.

The description is identical to that of *Haloterrigena thermotolerans* as given previously (Montalvo-Rodriguez et al., 2000) with the amendments as follows. Cells are 0.4–1.0 × 1.0–13.0 µm. Aerobic growth occurs in the presence of 10–30% (w/v) NaCl, pH 6.5–8.5 and 17–60°C. Optimal NaCl concentration and temperature for growth are 15–20% (w/v) and 37–50°C, respectively. Anaerobic growth in the presence of nitrate is variable. Oxidase activity, reduction of nitrate to nitrite and indole formation are variable. Hydrolysis of casein and gelatin is variable. Assimilation of fructose and lactose as carbon and energy sources is variable. The DNA G + C content is 65.0–65.4 mol% (genome).

The type strain is PR5T (= ATCC 700275T = DSM 11552T). The G + C content of the type strain genome is 65.4 mol%, its approximate size 3.90 Mb, and its GenBank Assembly accession number is GCA_000337115.1.

*Haloterrigena jeotgali* A29 (= CECT 7218 = DSM 18794 = JCM 14585 = KCTC 4020) is an additional strain of *Natrinema thermotolerans*. The G + C content of this reference strain genome is 65.0 mol%, its approximate size 4.90 Mb, and its GenBank Assembly accession number is GCA_004799625.1.

**Description of Natrinema salifodinae comb. nov.**

*Natrinema salifodinae* (sa.li.fo.di‘nae. N.L. gen. fem. n. salifodinae of a saltpit, salt mine)

Basonym: *Halopiger salifodinae* Zhang et al., 2013, 3565.

The description is identical to that of *Halopiger salifodinae* as given previously (Zhang et al., 2013) with the following amendments: the G + C content of the type strain genome is 65.4 mol%, its approximate size 4.27 Mb, and its GenBank Assembly accession number is GCA_900110455.1.

The type strain is KCY07-B2T (= CGMCC 1.12284T = DSM 26231T = JCM 18547T).

**Description of Natronorubrum daqingense comb. nov.**

*Natronorubrum daqingense* (da.qing.en’se. N.L. neut. adj. daqingense pertaining to Daqing, north-east China, where the type strain was isolated)

Basonym: *Haloterrigena daqingensis* Wang et al., 2010, 2270.

The description is identical to that of *Haloterrigena daqingensis* as given previously (Wang et al., 2010) with the following amendments: the G + C content of the type strain genome is 61.3–61.4 mol%, its approximate size 3.83–3.84 Mb, and its GenBank Assembly accession numbers are GCA_900156445.1 and GCA_001971705.1.

The type strain is JX313T (= CGMCC 1.8909T = NBRC 105739T).

**Emended description of the genus Natrinema**

*Natrinema* (Na.tri.ne‘ma. N.L. n. natrium sodium; Gr. neut. n. nema a thread; N.L. neut. n. *Natrinema* the sodium thread, referring to the high sodium ion requirement, and the cell shape)

Cells are rods, coccoid or pleomorphic. Cells lye at low NaCl concentration (<1.0 M). Colonies are red, light
orange-red, pale orange-red, or cream pigmented. Chemo-organotroph. Some species are strict aerobes, whereas others show anaerobic growth with nitrate. Catalase positive. Grows on a wide range of substrates, including single and complex carbon sources. Extremely halophilic, requiring at least 9–10% (w/v) NaCl for growth, with optimum at 15–29% (w/v) NaCl. Grows at pH values of 5.5–9.0, with optimum pH at 6.0–8.2. Temperature supporting growth ranges from 17 to 61°C, with optimum at 20–55°C. Possesses C20C20 and C20C25 diether core lipids. The major polar lipids consist of phosphatidylglycerol and phosphatidylglycerol-phosphate-methyl ester, with some species also containing phosphatidylglycerol sulfate. Most species possess the glycolipid S2-DGD-1, while some species possess S-DGD-1 or unidentified glycolipids. The DNA G + C content is in the range of 60.7–65.4 mol% (genome). The genus is a member of the family Natrálbaceae, order Natrálbales, class Halobacteria. The recommended three-letter abbreviation is Nnm. The type species is Natrinema pellirubrum.

Emended description of the genus Haloterrigena

Haloterrigena (Ha.lo.ter.ri'ge.na. Gr. n. hals halos the sea, salt; L. fem. adj. terrigena born from the earth; N.L. fem. n. Haloterrigena salt (-requiring) and born from the earth).

Cells are Gram-strain-negative, coccoid, or oval-shaped, and 1.1–2.0 μm in size. Colonies are colored light red or light pink due to the presence of bacterioruberin carotenoids. Cells are non-motile or motile and aerobic. Catalase-positive and oxidase-variable. Extremely halophilic, with growth occurring in media containing 10–30% (w/v) NaCl, with optimum at 15–25% (w/v) NaCl. Cells lyse in distilled water. Species may require or not magnesium to grow. Grows at pH values of 6.0–9.5, with optimum pH at 7.0–8.0. Temperature supporting growth ranges from 20 to 55°C, with optimum at 37–45°C. Some species reduce nitrate to nitrite but they do not form gas from nitrate. Indole formation and H2S production are negative. Hydrolysis of starch, gelatin and Tween 80 is negative. Chemo-organotrophic. All species use sugars, some of them with the production of acids. The major polar lipids are C20C20 and C20C25 glycerol diether derivatives of phosphatidylglycerol and phosphatidylglycerol-phosphate-methyl ester as well as the glycolipid S2-DGD-1. Some species may also contain the glycolipid S-DGD-1. Phosphatidylglycerol sulfate is absent. The DNA G + C content is between 64.5 and 65.4 mol% (genome). The genus is a member of the family Natrálbaceae, order Natrálbales, class Halobacteria. The recommended three-letter abbreviation is Nrr. The type species is Natronorubrum bangense.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

RRH, HM, MK,YS, and AV: conceptualization, investigation, writing – review and editing. RRH, HM, and YS: methodology, formal analysis. RRH, HM, and MK: validation. HM, MK, YS, and AV: resources, project administration, and funding acquisition. RRH and HM: data curation, writing – original draft preparation, and visualization. MK, YS, and AV: supervision. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.740909/full#supplementary-material
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