Progress in Biological Sciences
Vol. 3, Number 2, Summer/Fall 2013/1-26

Inventory of new microbial taxa from Iran

Received: November 10, 2013; Accepted: November 24, 2013

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Abstract

Microorganisms constitute more than half of the Earth's biomass and in addition to quantity, they represent great diversity. In January 2013, the number of officially registered names for prokaryotic microorganisms at the genus and species levels were, respectively, approximately 2100 and 12000. Current authorized methods for classification and identification of new microbial taxa are polyphasic approaches which make use of a codified set of characteristics for classification of microorganisms. Here, we present a brief overview of criteria by which prokaryotes are classified and subsequently summarize some recent findings on the microbial flora of Iran. Among all microbial taxa identified to date, six taxa at the genus level and 24 taxa at the species level were first discovered in and reported from Iran. This attests to the existence of a rich microbial diversity in Iran. Among the species, four belong to the *Archaea* and the others belong to the *Bacteria* domain. The bacterial taxa consist of six Gram- negative species within *Proteobacteria* and *Bacteroidetes* phylems and 20 Gram-positive taxa that are among the *Actinobacteria*, *Firmicutes* and related organisms. It is incumbent that further focus be placed on the rich ecological diversity of Iran, and it is expected that this will result in identification of new endemic microorganisms.

Key Words: characterization, identification, Iran, microbial diversity, polyphasic approach, taxonomy.

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Introduction

"Taxonomy... is the most subjective branch of any biological science, and in many ways is more of an art than a science".

S.T.Cowan, 1965

For much of the last century, microbiologists were aware that we know the identity of only a tiny fraction of the inhabitants of the prokaryotic world. While scientists are very familiar with the diversity of life in the plant and animal kingdoms, only some realize the vast amount of variability present in *prokaryptic* populations. The total number of microbial cells on the Earth is estimated to be 10^{30}, comprising 10^6 to 10^8 separate genospecies. Microorganisms represent the richest repertoire of molecular and chemical diversity in nature, and they participate in basic ecosystem processes. The current inventory of the world's prokaryotic diversity is very incomplete and particularly deficient. Studies indicate that the 12000 identified species of prokaryotes represent only a small percent of all bacterial species; therefore, we have only a small idea of our true microbial diversity (1).

Taxonomy is the science of classification of organisms. Bacterial taxonomy consists of three separate, but interrelated endeavors: classification, nomenclature, and identification. Classification is the arrangement of organisms into groups (taxa) on the basis of similarities or relationships. Nomenclature is the assignment of names to the taxonomic groups in accordance with the rules of the *International Code of Nomenclature of Bacteria* (2). Identification is the practical use of a classification with the objective of determining the identity of an isolate as a member of an already established taxon or as a member of previously unidentified taxa.

The some 12000 bacterial species thus far described great diversity, and the tens of thousands of postulated species that remain to be described are expected to add to the known diversity. Classification has been used to organize an array of individual bacteria into an orderly framework. Adequate description of bacteria and classification require knowledge of their morphologic, biochemical, physiological, and genetic characteristics. As a science, taxonomy is dynamic and subject to change on the basis of available data. The classification of prokaryotic organisms has undergone many changes since microbial life was first described. All of these changes rested on data acquired by application of technological advances. New instruments, techniques and analysis protocols were very important in the development of the field of microbiology and, in particular, microbial systematic (3). The history of microbial taxonomy started with discovery of microorganisms. Later, the advent of pure cultures allowed description of many phenotypic features that served as additional criteria for classification. The introduction of molecular analysis protocols, particularly gene sequencing and genomics had profound effects on the field. Today with the advent of next generation sequencing technologies and bioinformatics platforms for total genome analysis, a revolution in microbial systematics is expected (4).

In the classification scheme, if the taxa have already been described, named, and classified, new characteristics may be added or existing characteristics may be reinterpreted, possibly resulting in updating, revision, or formulation of new classifications. But if the organism is new, i.e. cannot be identified as a member of an existing taxon, it is named and described according to the rules of nomenclature and placed in an appropriate position in the existing classification scheme.

Taxonomic ranks

Several ranks are used in bacterial classification. The highest rank is called a Domain. All prokaryotic organisms are placed within one of two Domains, *Archaea* and *Bacteria*. Phylum, class, order, family, genus, species, and subspecies are successively subsets of the Domain. The names of these subsets from class to subspecies are given formal recognition. At present, neither kingdoms nor divisions are used in the classification scheme of prokaryotes.

Species

The basic and most important taxonomic group in bacterial systematics is the species, much as in eukaryotic organisms. The concept of a bacterial species is less definitive than for higher organisms. A microbial species concept could provide a way to organize and make sense of prokaryotic diversity and enable communication of information, but it is a controversial topic.

Today, species are defined by pragmatic and sometimes artificial methods in a formalized polyphasic approach based on genomic data including 16S ribosomal RNA (rRNA) sequences, genomic similarity as evidenced by at least 70% hybridization of their DNAs (DNA-DNA hybridization; DDH) and phenotypic data including morphology, physiology, and chemotaxonomy. A prokaryotic species is considered to be a group of strains that are characterized by a certain degree of phenotypic consistency, showing 70% of DNA–DNA hybridization under standard conditions, and over 97% of 16S rRNA gene-sequence similarity (5). This system is functional in many ways, and the authorized species categories provide a common dialect that scientists and practitioners can use to convey information. Taxonomists can incorporate new knowledge into a species definition at any stage as new techniques become available, and there are mechanisms for incorporating novel data that may be beneficial to the users of species descriptions. The fixed rules and cut-offs in use today for prokaryotic taxonomy are inadequate. Most recently, whole-genome sequencing has delivered new taxonomic metrics—for example, average nucleotide identity (ANI), calculated from pair-wise comparisons of all sequences shared between any two strains. ANI exhibits a strong correlation with DDH values, with an ANI value of $\geq 95\%$ corresponding to the traditional 70% DDH threshold (6).The microbial species definition is drawn from data on a limited sample of organisms specially from those that we can retrieve by culture dependent methods, but it is extrapolated to the entirety of the microbial world. There are some suggestions which provide that the phylogenomic species concept, which combines phylogenetic and genomic analyses, can be used to circumscribe species. Phylogenetic analyses of 16S rRNA sequences are currently used to ascertain the taxonomy of Bacteria and Archaea at higher taxonomic levels, and less highly conserved genes can be used for species. Horizontal gene transfers pose a major challenge for any taxonomy, but genomic approaches will help resolve this issue. The phylogenomic species concept could apply universally to all organisms (7).

Type strain

One strain of a species is designated as the type strain; this strain serves as the name-bearer strain of the species and is the reference example of the species. The type strain has great importance for classification at the species level, because a species consists of

the type strain and all other strains that are considered to be sufficiently similar to it as to warrant inclusion with it as members of a single species. Any strain can be designated as the type strain, although, for new species, the first strain isolated is usually designated as the type strain (8).

Polyphasic taxonomy

Taxonomy is generally considered as a synonym of systematics or biosystematics. As already stated, it is traditionally divided into three parts: classification, nomenclature and identification of unknown organisms (9,10). Two additional concepts are needed to completely define modern biosystematics: phylogen and population genetics. We believe that bacterial classification should reflect as closely as possible the natural relationships between bacteria. Phylogenetic relationships based on 16S or 23S rRNA sequence data reflect an attempt to achieve this goal (11). Population genetics and total cell genomics data will be of a great help in this field.

The term "polyphasic taxonomy" was introduced 43 years ago by Colwell (1970) to refer to a taxonomy that considers many levels of information and incorporates several distinct, and separate portions of information extractable from a nonhomogeneous system to yield a multidimensional taxonomy (12). Nowadays, polyphasic taxonomy refers to a consensus type of taxonomy and aims to utilize all the available data and get a multidimensional view for arranging microorganisms in separate groups.

The species is the basic unit of bacterial taxonomy, and the first recommendation for a polyphasic consensus delineation of a bacterial species is based on "the phylogenetic species definition" of Wayne et al. (1987). Total genome DNA–DNA hybridization values is the key parameter in this species delineation which defined a species as a group of strains, including the type strain, sharing at least 70% total genome DNA–DNA hybridization and less than 5ºC ΔTm (13).

What types of information do we use in Polyphasic Taxonomy?

To have a better multidimensional view for taxonomy both genotypic and phenotypic information may be incorporated. The information that we use for polyphasic taxonomy can come from diverse techniques which are represented schematically in Figure 1. The ultimate characterization on the genomic level is the determination of the sequence and mapping the total bacterial genome. As long as this cannot be performed routinely, the best way to collect a maximum amount of direct and indirect information about the total genome is a polyphasic approach. Each method in polyphasic taxonomy has a different taxonomic resolution which is very important in the taxonomic levels that this method can make discriminable. Figure 2 presents the discriminatory taxonomic power of the techniques summarized in Figure 1.

Genotypic Methods

Genotypic methods are those that are directed toward DNA or RNA molecules. Our present view on classification is that it should reflect the natural relationships as encoded in the DNA.

Determination of the DNA base ratio (moles percent G+C)

Determination of the moles percent guanine plus cytosine is one of the classic genotypic methods and is still part of the standard description of bacterial taxa. This having been said, it is now considered less informative

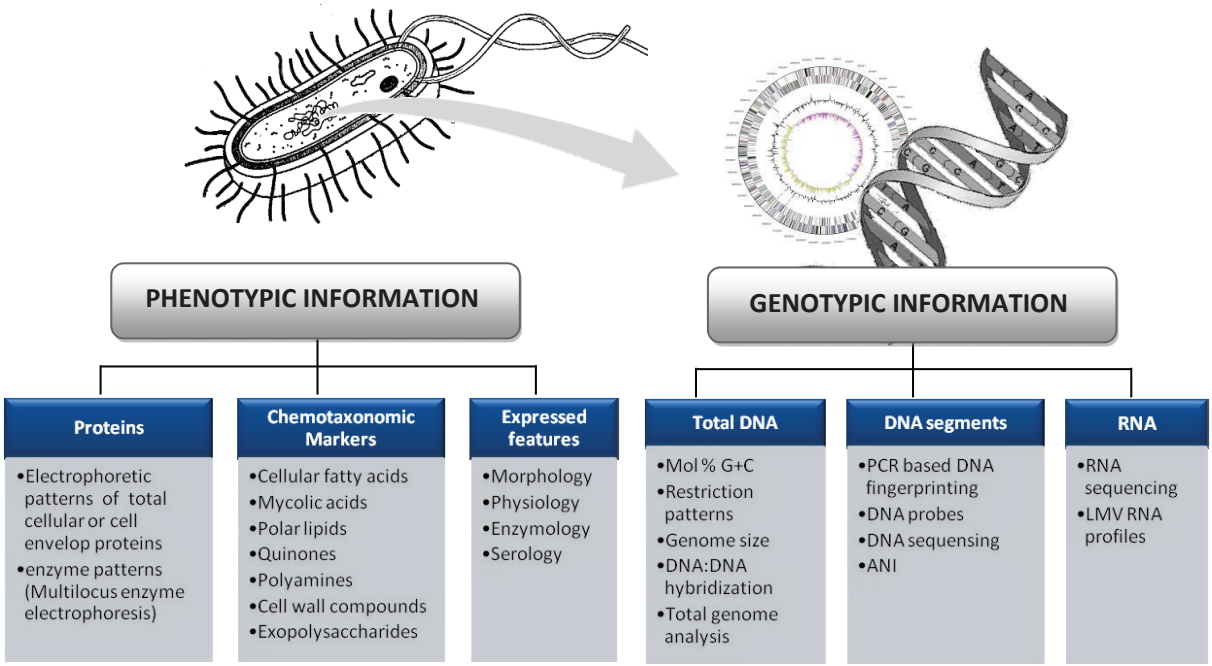

Figure 1. Schematic overview of various cellular components and techniques used in polyphasic bacterial taxonomy. (Adapted from Vandamme *et al.*, 1996.)

than the genotypic methods later developed. Generally, the range of observed is not more than 3% difference within a well-defined species and not more than 10% difference within a well-defined genus (14). It varies between 24% and 76% in the bacterial world.

DNA-DNA hybridization studies

The percent DNA-DNA hybridization and the decrease in thermal stability of the hybrid are used to delineate species (13). The percent DNA binding (15) or the DNA-DNA hybridization value or the relative binding ratio (16) is an indirect parameter of the sequence similarity between two entire genomes. It has been established that thermal stabilities decrease from 1 to 2.2 degrees for each 1% of mispairing (17, 18).

rRNA homology studies

It is now generally accepted that rRNA is the best target for studying phylogenetic relationships because it is present in all bacteria, is functionally constant, and is composed of highly conserved as well as more variable domains (11,17,19). Nowadays direct sequencing of nearly entire 16S RNA or a part of 23S RNA molecules is a routine laboratory technique and provides a phylogenetic framework which serves as the backbone for modern microbial taxonomy.

Phenotypic methods

Phenotypic methods comprise all those that are not directed toward DNA or RNA. They include the chemotaxonomic techniques. Classical phenotypic methods analyze morphologic, physiologic, and biochemical features of prokaryotic cells. In chemotaxonomy, more informative chemical constituents of prokaryotic cells, such as protein and lipid components of cell walls

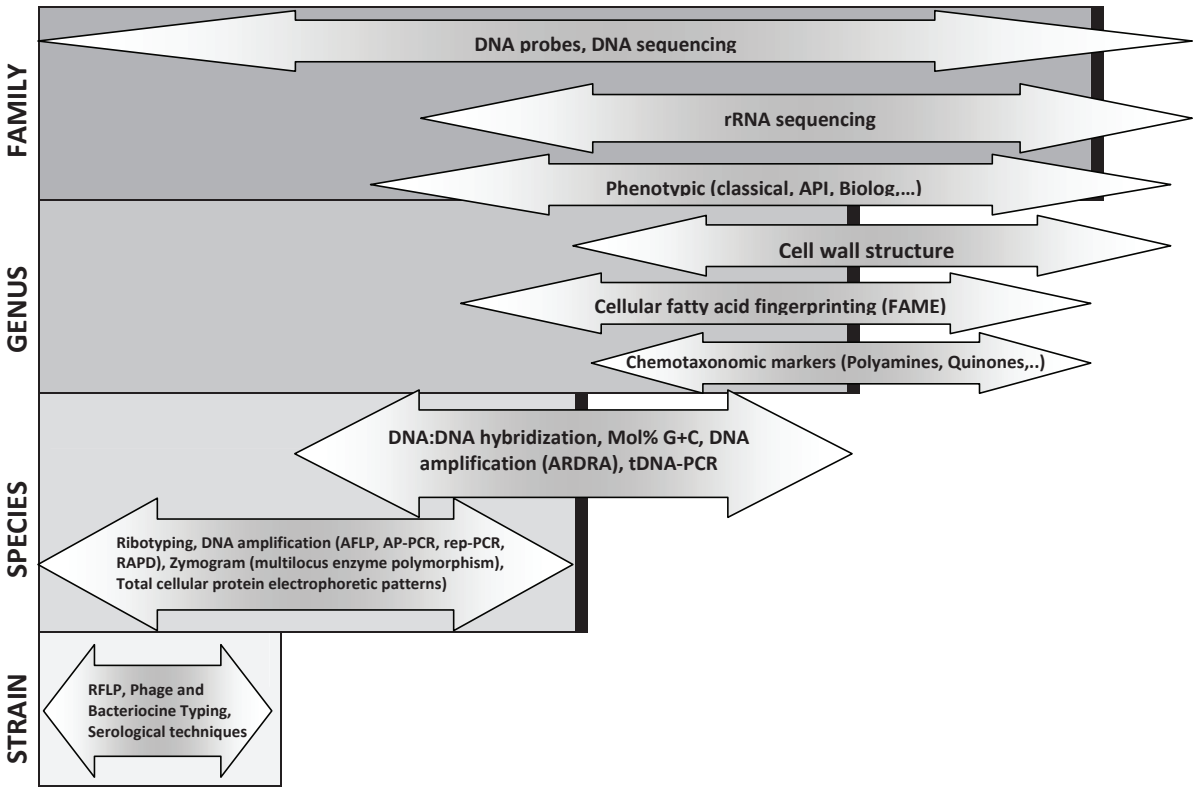

Figure 2. Taxonomic resolution of some of the currently used techniques in bacterial taxonomy. (Adapted from Vandamme *et al.*, 1996.) Abbreviations: AFLP, amplified fragment length polymorphism; AP-PCR, arbitrarily primed PCR; ARDRA, amplified rDNA restriction analysis; FAMEs, fatty acid methyl esters; LMW, low molecular weight; PFGE, pulsed-field gel electrophoresis; RAPD, randomly amplified polymorphic DNA; rep-PCR, repetitive element sequence-based PCR; RFLP, restriction fragment length polymorphism; 1D, 2D, one and two-dimensional, respectively.

are analyzed and compared. As for the other phenotypic and the genotypic techniques, the various chemotaxonomic methods differ in power of resolution; some are widely used and some only have very specific applications.

Classical phenotypic analyses

The classical phenotypic tests are used in identification schemes in the majority of microbiology laboratories. They constitute the common basis for the formal description of taxa, from species and subspecies up to genus and family. While genotypic data are used to allocate taxa on a phylogenetic tree and to draw the major borderlines in classification systems, phenotypic consistency is required to generate useful classification systems and may therefore influence the depth of a hierarchical line. The morphology of a bacterium includes both cellular (shape, endospore, flagella, inclusion bodies, Gram staining) and colonial (color, dimensions, form) characteristics. The physiological and biochemical features include data on growth at different temperatures, pH values, salt concentrations, or atmospheric conditions, growth in the presence of various substances such as antimicrobial agents, and data on the presence or activity of various

enzymes, metabolization of compounds, etc. Very often, highly standardized procedures are required to obtain reproducible results within and between laboratories (20, 21).

Miniaturized commercial phenotypic fingerprinting systems such as Biolog or API system have been introduced and may in the future replace commonly used laboratory protocols for phenotypic analyses. These systems generally include standardized reagents and standardized analysis protocols. These features enable maximum consistencies between results from different laboratories. The systems have significantly contributed to obtaining consistent results and reliable information on prokaryotic taxonomy.

Chemotaxonomy analysis

Cell wall composition

Determination of the cell wall composition has traditionally been important in Gram-positive bacteria. The peptidoglycan type of Gram-negative bacteria is rather uniform and provides little information. Cell walls of Gram-positive bacteria, in contrast, contain various peptidoglycan types, which may be genus or species specific (22). Membrane-bound teichoic acid is present in all Gram-positive species (23), whereas cell wall-bound teichoic acid is present in only some Gram-positive species (24).

Cellular fatty acids

A variety of lipids are present in bacterial cells. Polar lipids are the major constituents of the lipid bilayer of bacterial membranes and have been studied frequently for classification and identification purposes. Other types of lipids, such as sphingophospholipids, occur in only a restricted number of taxa and were shown to be valuable tools within these groups (25). The lipopolysaccharides present in the outer membranes of Gram-negative bacteria can be analyzed by gel electrophoresis, giving typical lipopolysaccharide ladder patterns which are interpreted as variants in the O-specific side chains (26,27). Fatty acids are the major constituents of lipids and lipopolysaccharides and have been used extensively for taxonomic purposes. More than 300 different chemical structures of fatty acids have been identified. The variability in chain length, double-bond position, and substituent groups has proven to be very useful for the characterization of bacterial taxa (28). Commonly, the total cellular fatty acid fraction is extracted, but particular fractions such as the polar lipids have also been analyzed (29). Cellular fatty acid methyl ester content is a stable parameter provided that highly standardized culture conditions are used. The method is rapid and has reached a high degree of automation.

Isoprenoid quinines

Isoprenoid quinones occur in the cytoplasmic membranes of most prokaryotes and play important roles in electron transport and oxidative phosphorylation (30,31). For these quinines two major structural groups, the naphthoquinones and the benzoquinones, are distinguished. The former can be further subdivided into two main types, the phylloquinones, which occur less commonly in bacteria, and the menaquinones. The large variability of the side chains (differences in length, saturation, and hydrogenation) can be used to characterize bacteria at different taxonomic levels (31).

Whole-cell protein analysis

The comparison of whole-cell protein patterns obtained by highly standardized sodium dodecyl sulfate-polyacrylamide gel

electrophoresis (SDS-PAGE) can provide discriminative information at or below the species level and it has been proven to be extremely reliable for comparing and grouping large numbers of closely related strains (32). Numerous studies have revealed a correlation between high similarity in whole-cell protein content and DNA-DNA hybridization (33).

Polyamines

Polyamines in the bacterial cell are important in bacterial metabolism (34). Depending on the group of organisms studied, polyamine patterning has been used to trace relatedness at and above the genus level (35,36).

New prokaryotic taxa from Iran

Amongst 2100 new valid name at the genus level and almost 12000 valid names at the species level in the prokaryotic world (37, http://www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up-to-date.html), so far six taxa at the genus level and 24 taxa at the species level have been introduced for the first time and classified from the rich and unique microbial diversity of Iran. The taxa are introduced below, brief review of their protolouges is presented. The three groups of Gram-positive and Gram-negative bacteria and archaea are all represented in the taxa from Iran.

Classified taxa in *Archaea* Domain from Iran

In *Archaea* domain 3 new taxa in genus level and 1 new taxa in species level have been introduced from Iran and all of them are extremely halophilic microorganisms isolated from Aran-Bidgol hypersaline Lake.

Halopenitus persicus gen. nov., sp. nov. protologue

Halopenitus persicus is a new species classified in new genus with DC30^T as type strain. Strain DC30^T was isolated from Aran-Bidgol salt lake. DC30^T had non-motile and pleomorphic cells. Analysis of the 16S rRNA gene sequence similarity showed that strain DC30^T was a member of the family *Halobacteriaceae*. However, it had low 16S rRNA gene sequence similarities to the most closely related haloarchaeal taxa. The DNA G+C content of this strain was 66.0 mol%. Phosphatidylglycerol and phosphatidylglycerol phosphate methyl ester, common phospholipids found in haloarchaea, were present. Three minor phospholipids and one unidentified glycolipid were also observed. The only quinone present was MK-8. The type strain of *Halopenitus persicus* is DC30^T with the accession number of IBRC 10041^T at Iranian Biological Resource Center (38).

Halopenitus malekzadehii sp. nov. protologue

A novel extremely halophilic archaeon, was isolated from a brine sample of Aran-Bidgol salt lake. The novel strain was light yellow pigmented and it has non-motile cells. Analysis of 16S rRNA gene sequence revealed that strain CC65^T belongs to the genus *Halopenitus*, with a sequence similarity of 98.0% to the type strain. The polar lipid pattern of strain CC65^T consisted of phosphatidylglycerol and phosphatidylglycerol phosphate methyl ester. An unidentified glycolipid and two minor phospholipids were also observed. The only quinone present was MK-8. The G+C content of its DNA was 63.8 mol%. Strain CC65^T showed 44% DNA-DNA hybridization with *Halopenitus persicus* IBRC 10041^T which conclude that with polyphasic characterization approach the new isolate could be classified

as a novel species of the genus *Halopenitus*, for which the name *Halopenitus malekzadehii* sp. nov. is proposed. Strain CC65^T is the type strain of *Halopenitus malekzadehii* with IBRC-M 10418^T accession number in Iranian Biological Resource Center (39).

Halovenus aranensis gen. nov., sp. nov. protologue

A novel red-pigmented halophilic archaeon was isolated from Aran-Bidgol salt lake. Cells of strain EB27^T were non-motile and pleomorphic. 16S rRNA gene sequence analysis showed that strain EB27^T is a member of the family *Halobacteriaceae*; however, levels of 16S rRNA gene sequence similarity to the most closely related haloarchaeal taxa were low. The DNA G+C content of strain EB27^T was 61 mol%. Strain EB27^T contained phosphatidylglycerol and phosphatidylglycerol phosphate methyl ester, common phospholipids found in haloarchaea, together with two minor phospholipids. The only quinone present was MK-8(II-H_2). Physiological, biochemical and phylogenetic differences between strain EB27^T and recognized genera of extremely

Table 1. Classified taxa in *Archaea* Domain from Iran in one look

	Criteria / Name	*Halopenitus persicus* DC30^T	*Halopenitus malekzadehii* CC65^T	*Halovenus aranensis* EB27^T	*Haloarchaeobius iranensis* EB21^T
Genomic data	16S rRNA sequence similarity with closely related taxa	92.4%: *Halorubrum* type species	98.0%: *Halopenitus* type species	90%: *Halalkalicoccus tibetensis* DS12^T	91.8%: *Halobiforma lacisalsi*
	G+C mol percent	66.0	63.8	61.0	67.7
	DNA:DNA hybridization value	–	44%	-	-
Chemotaxonomic data	Polar lipid	Phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, Three minor phospholipids,one unidentified glycolipid	Phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, An unidentified glycolipid, two minor phospholipids	Phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, two minor phospholipids	phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester , phosphatidylglycerol sulfate, Three unidentified glycolipids, one minor phospholipid
	Quinone	MK-8	MK-8	MK-8	MK-8
Morphologic data	Cell shape	Pleomorphic	Pleomorphic	Pleomorphic	Rod
	PSigmentation	Pale pink	Light yellow	Red	Orange–red
	Motility	Non-motile	Non-motile	Non-motile	Motile
Physiologic data	Optimum NaCl for growth (M)	3	3.5	4	3.5
	Optimum pH for growth	7.5	7.5	7.5	7.5
	Optimum Temperature for growth (°C)	40	40	40	40
	Accession number	IBRC 10041^T	IBRC-M 10418^T	IBRC-M 10015^T	IBRC-M 10013^T

halophilic archaea suggest that this strain represents a novel species in a new genus within the family *Halobacteriaceae*, for which the name *Halovenus aranensis* gen. nov., sp. nov. is proposed. The type strain of *Halovenus aranensis* and the type species of the new genus, is strain EB27^T which have been deposited in Iranian Biological Resource Center with IBRC-M 10015^T accession number and The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EB27^T is HQ19798 (40).

Haloarchaeobius iranensis gen. nov., sp. nov. protologue

Strain EB21^T was isolated from a brine sample from Aran-Bidgol salt lake. Strain EB21^T was an orange–red-pigmented, motile rod and required at least 2 M NaCl but not $MgCl_2$ for growth. Analysis of the 16S rRNA gene sequence revealed that strain EB21^T is a member of the family *Halobacteriaceae*, showing low levels of similarity to other members of the family. Polar lipid analyses revealed that strain EB21^T contains phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and phosphatidylglycerol sulfate. Three unidentified glycolipids and one minor phospholipid were also observed. The only quinone present was MK-8. The G+C content of its DNA was 67.7 mol%. On the basis of the data obtained Strain EB21^T is considered to represent a novel species in a new genus within the family *Halobacteriaceae*, order *Halobacteriales*, for which the name *Haloarchaeobius iranensis* gen. nov., sp. nov. is proposed. The type strain of *Haloarchaeobius iranensis* is EB21^T (IBRC-M 10013^T). The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain EB21^T are JF293278 (*rrnA*) and JF293279 (*rrnB*) (41).

Classified taxa in Gram-negative bacteria from Iran

Salinivibrio proteolyticus sp. nov. protologue

A novel moderately halophilic, Gram-negative, curved-rod-shaped bacterium, designated strain AF-2004^T, was isolated from Bakhtegan Lake. Strain AF-2004^T was a facultative anaerobe, motile by one polar flagellum, non-sporulating and oxidase- and catalase-positive. Phylogenetic analyses based on 16S rRNA gene sequence similarity indicated that strain AF-2004^T is a member of the genus *Salinivibrio*. Strain AF-2004^T had $C_{18:1}\omega7c$ (31.6%), $C_{16:1}\omega7c$ (22.1%) and $C_{16:0}$ (20.7%) as the predominant fatty acids and Q-8 as the major respiratory lipoquinone. The DNA G+C content was 49.5 mol%, which is in the range of values for members of the genus *Salinivibrio*. On the basis of phenotypic and chemotaxonomic characteristics, 16S rRNA sequence analysis and DNA–DNA relatedness, it is proposed that strain AF-2004^T (=DSM 19052^T =CIP 109598^T) should be placed in the genus *Salinivibrio* as the type strain of a novel species, *Salinivibrio proteolyticus* sp. nov. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AF-2004^T is DQ092443 (42).

Limimonas halophila gen. nov., sp. nov. protologue

A novel, Gram-staining-negative, extremely halophilic bacterium, designated strain IA16^T, was isolated from the mud of the hypersaline Lake Aran-Bidgol. Cells of strain IA16^T were not motile, non-pigmented, rod-shaped, and strictly aerobic. Phylogenetic analysis based on 16S rRNA gene sequences similarity indicated that strain IA16^T belonged in the

family *Rhodospirillaceae*. The novel strain's major cellular fatty acids were $C_{19:0}$ cyclo ω7c and $C_{18:0}$ and its polar lipid profile comprised phosphatidylglycerol, diphosphatidylglycerol, four unidentified phospholipids, three unidentified aminolipids and two other unidentified lipids. The cells of strain IA16^T contained the ubiquinone Q-10. Strain IA16^T showed 67.0 mol% of G+C in its genomic DNA. On the basis of physiological, biochemical and phylogenetic differences it is concluded that the strain represents a novel species in a new genus within the family *Rhodospirillaceae*, for which the name *Limimonas halophila* gen. nov., sp. nov. is proposed. The type strain of *Limimonas halophila* is IA16^T with IBRC-M 10018^T accession number. The GenBank/ EMBL/DDBJ accession number for the

Table 2. Classified taxa in Gram-negative bacteria from Iran in one look

Criteria	Name	*Salinivibrio proteolyticus* AF-2004^T	*Limimonas halophila* IA16^T	*Alishewanella tabrizica* RCRI4^T	*Salinibacter iranicus* CB7^T	*Salinibacter luteus* DGO^T	*Marinobacter persicus* M9B^T
Genomic data	16S rRNA sequence similarity with closely related taxa	99.0%: *Salinivibrio costicola* subsp. *vallismortis* DSM 8285^T	91.6%: *Rhodovibrio sodomensis* DSM 9895^T	97.8%: *Alishewanella agri* BLO6^T	93.2%: *Salinibacter ruber* DSM 13855^T	93.6%: *Salinibacter ruber* DSM 13855^T	97.7%: *Marinobacter hydrocarbonoclasticus* MBIC 1303^T
	G+C mol percent	49.5	67.0	45.3	64.8	65.6	58.6
	DNA:DNA hybridization value	10.1	-	9	44	52	13±2%
Chemotaxonomic data	Polar lipid	-	phosphatidylglycerol, diphosphatidylglycerol, four unidentified phospholipids, three unidentified aminolipids, two other unidentified lipids	-	diphosphatidylglycerol, phosphatidylcholine, three unidentified lipids, one unidentified aminolipid, three unidentified glycolipids	diphosphatidylglycerol, phosphatidylcholine, three unidentified lipids, one unidentified aminolipid, three unidentified glycolipids	phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine , three phospholipids
	Quinone	Q-8	Q-10	-	MK-7	MK-7	Ubiquinone 9
	Predominant fatty acid	$C_{18:1}\omega7c$ (31.6%), $C_{16:1}\omega7c$ (22.1%), $C_{16:0}$ (20.7%)	$C_{19:0}$ cyclo $\omega7c$ and $C_{18:0}$	$C_{17:0}$, $C_{16:1}\omega7c/C_{15}$ iso3-OH, $C_{17:1}\omega8c$, $C_{16:0}$	$C_{18:1}\omega7c$, iso-$C_{15:0}$, $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH	$C_{18:1}\omega7c$, iso-$C_{15:0}$, $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH	C16:0, C19:1 ω6c, C18:1 ω9c and C16:1 ω9c
Morphologic data	Cell shape	Curved-rod	Rod	Rod	Rod	Rod	Rod
	Gram- staining	Gram-negative	Gram-negative	Gram-negative	Gram-negative	Gram-negative	Gram-negative
	Pigmentation	Creamy white	Non-pigmented	Pallid	Red	Orange	Orange-yellow
	Motility	Motile	Non-motile	Motile	Non-motile	Non-motile	Motile
Physiologic data	Optimum NaCl for growth (% w/v)	5	17	Able to grow in the absence and presence of NaCl to 3%	15	15	7.5-10
	Optimum pH for growth	8.0–8.5	7.0	7±0.5	7.5	7.5	7.0
	Optimum Temperature for growth (°C)	32.0–35.0	40	30	37	37	35
Accession number		DSM 19052^T	IBRC-M 10018^T	KCTC 23723^T	IBRC-M 10036^T	IBRC-M 10423^T	IBRC-M 10445^T

16S rRNA gene sequence of strain IA16^T is JN605361 (43).

Alishewanella tabrizica sp. nov., protologue

A novel Gram-negative bacteria, RCRI4^T, was isolated from Qurugöl Lake located in a mountainous region near Tabriz city. The cells of RCRI4^T strain were aerobic, motile and rod-shape. The major fatty acids of the novel strain were $C_{17:0}$, $C_{16:1}\omega7c$/C15 iso3-OH, $C_{17:1}\omega8c$ and $C_{16:0}$. The G+C content of genomic DNA was 45.3 mol%. Based on the 16S rRNA and *gyrB* gene sequences similarity, phylogenetic analyses indicated that strain RCRI4^T associated with the genus *Alishewanella*. The level of DNA–DNA relatedness between strains RCRI4^T and phylogenetically the closest related strains were at the range to announce RCRI4^T as a new member of *Alishewanella* genus. On the basis of phenotypic, chemotaxonomic and phylogenetic results, it is suggested that strain RCRI4^T represents a novel species of the genus *Alishewanella*, for which the name *Alishewanella tabrizica* sp. nov. is proposed. The type strain is RCRI4^T (=LMG 26473^T=JCM 17275^T=KCTC 23723^T). The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and partial *gyrB* gene sequences of strain RCRI4^T are GQ505294 and JN106465, respectively (44).

Salinibacter iranicus sp. nov. and *Salinibacter luteus* sp. nov. protologue

Two Gram-staining-negative and extremely halophilic bacteria, designated strains CB7^T and DGO^T, were isolated from Aran-Bidgol hypersaline Lake. The predominant fatty acids of the two isolates were $C_{18:1}\omega7c$, iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH). The polar lipid pattern of the two isolates consisted of diphosphatidylglycerol, phosphatidylcholine, three unidentified lipids, one unidentified aminolipid and three unidentified glycolipids. The only quinone present was menaquinone 7 (MK-7). The G+C contents of the genomic DNA of strains CB7^T and DGO^T were 64.8 and 65.6 mol%, respectively. 16S rRNA gene sequence similarity analysis indicated that strains CB7^T and DGO^T were related to *Salinibacter ruber* in the phylum *Bacteroidetes* with low levels of gene sequence similarity. The two novel strains shared 98.9% 16S rRNA gene sequence similarity. DNA–DNA hybridization experiments between strains CB7^T and DGO^T and *Salinibacter ruber* DSM 13855^T indicated levels of relatedness of 44 and 52%, respectively, while the level of relatedness between the two new isolates was 53%. Chemotaxonomic data supported the placement of strains CB7^T and DGO^T in the genus *Salinibacter*. On the basis of DNA–DNA hybridization studies and biochemical and physiological characterization strains CB7^T and DGO^T are considered to represent two novel species of the genus *Salinibacter*, for which the names *Salinibacter iranicus* sp. nov. (type strain CB7^T=IBRC-M 10036^T) and *Salinibacter luteus* sp. nov. (type strain DGO^T=IBRC-M 10423^T) are proposed. Emended descriptions of the genus *Salinibacter* and of *Salinibacter ruber* are also presented. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CB7^T and DGO^T are HQ197982 and HQ197983, respectively (45).

Marinobacter persicus sp. nov. protologue

A Gram-negative and moderately halophilic bacterium, designated strain M9B^T, was isolated from the Aran-Bidgol hypersaline Lake. Cells of strain M9B^T were found to be non-endospore-forming, rod shaped, strictly

aerobic, motile and produce colonies with an orange-yellow pigment. Phylogenetic analysis based on the comparison of 16S rRNA gene sequences revealed that strain M9B^T is a member of the genus *Marinobacter*. DNA-DNA hybridization between the novel isolate and phylogenetically related species was 13 ± 2 %. The major cellular fatty acids of the isolate were identified as $C_{16:0}$, $C_{19:1}$ ω6c, $C_{18:1}$ ω9c and $C_{16:1}$ ω9c. The polar lipid pattern of strain M9B^T was determined to consist of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine and three phospholipids. Ubiquinone 9 was the only lipoquinone detected. The G+C content of the genomic DNA of this strain was determined to be 58.6 mol%. Phenotypic characteristics, phylogenetic analysis and DNA-DNA relatedness data suggest that this strain represents a novel species of the genus *Marinobacter*, for which the name *Marinobacter persicus* sp. nov. is proposed. The type strain of *Marinobacter persicus* is strain M9B^T with accession number IBRC-M 10445^T (46).

Classified taxa in Gram-positive bacteria from Iran

Saliterribacillus persicus gen. nov., sp. nov. protologue

Strain X4B^T a moderately halophilic bacteriumwas isolated from soil around the hypersaline lake Aran-Bidgol and characterized taxonomically using a polyphasic approach. Cells of strain X4B^T were Gram-positive, motile rods and formed ellipsoidal endospores at a terminal or subterminal position in swollen sporangia. Strain X4B^T was a strictly aerobic bacterium, catalase- and oxidase-positive. Analysis of 16S rRNA gene sequence revealed that strain X4B^T is a member of the family *Bacillaceae*, constituting a novel phyletic lineage within this family. The DNA G+C content of this novel isolate was 35.2 mol%. The major cellular fatty acids of strain X4B^T were anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ and its polar lipid pattern consisted of diphosphatidylglycerol, phosphatidylglycerol, two aminolipids, an aminophospholipid and an unknown phospholipid. The isoprenoid quinones were MK-7 (89%) and MK-6 (11%). The peptidoglycan contained *meso*-diaminopimelic acid as the diagnostic diamino acid. On the basis of 16S rRNA gene sequence analysis, chemotaxonomic and phenotypic data, strain X4B^T represents a novel species in a new genus in the family *Bacillaceae*, order *Bacillales* for which the name *Saliterribacillus persicus* gen. nov., sp. nov. is proposed. The type strain of the type species (*Saliterribacillus persicus*) is X4B^T which has been deposited in Iranian Biological Resource Center with IBRC-M 10629^T accession number. The GenBank/ EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Saliterribacillus persicus* strain X4B^T is HQ433437 (47).

Alteribacillus bidgolensis gen. nov., sp. nov. protologue

Strain P4B^T a moderately halophilic bacterium was isolated from water of the hypersaline Aran-Bidgol lake. Cells of strain P4B^T were Gram-positive, non-motile rods producing ellipsoidal endospore at a central position in non-swollen sporangia. Strain P4B^T was strictly aerobic and catalase- and oxidase-positive. On the basis of 16S rRNA gene sequence analysis, strain P4B^T was shown to belong to the phylum *Firmicutes* and shared highest similarity with *Bacillus persepolensis* HS136^T (97.1%) and *Bacillus salarius* BH169^T (95.1%). However, it shared only 91.3% 16S rRNA gene sequence similarity with *Bacillus subtilis* subsp. *subtilis* DSM 10^T, indicating that strain P4B^T might

Table3. Classified taxa in Gram-positive bacteria from Iran in one look

Name / Criteria	Genomic data			Chemotaxonomic data			Morphologic data	Physiologic data			Accession number
	16S rRNA sequence similarity with closely related taxa	G+C mol%	DNA:DNA hybridization value	Polar lipid	Quinone	Predominant fatty acid	Cell shape	Optimum NaCl for growth (% w/v)	Optimum pH for growth	Optimum Temperature for growth (°C)	
Saliterribacillus persicus X4B^T	96.0%: *Sediminibacillus albus*	35.2	-	DPG, PG, two AL, an APL,an unknown PL	MK-7, MK-6	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$	Rod	7.5	7.0	35	IBRC-M 10629^T
Alteribacillus bidgolensis P4B^T	97.1%: *Bacillus persepolensis* HS136^T	38.9	6%	PG, an AGL and an unknown PL	MK-7, MK-8	iso-$C_{15:0}$, anteiso-$C_{15:0}$	Rod	5–7.5	7.0	35	IBRC-M 10614^T
Alteribacillus persepolensis HS136^T	95.2%: *Bacillus salarius* BH169^T	37.1	-	PG, DPG	MK-7	iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$	Rod	10	8.0–8.5	40	DSM 21632^T
Ornithinibacillus halophilus G8B^T	97.6%: *Ornithinibacillus bavariensis* WSBC 24001^T	36.9	6%	PG, DPG, four unknown PL, an unknown AL	MK-7, MK-8	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$	Rod	5–7.5	7.5–8.0	35–40	IBRC-M 10683^T
Lentibacillus persicus Amb31^T	96.8%: *Lentibacillus salicampi* SF-20^T	44.1	-	PG, DPG, five PL, a GL	MK-7	anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$	Rod	7.5–10	7.5	30–35	DSM 22530^T
Bacillus iranensis X5B^T	95.6%*Bacillus persepolensis* HS136^T	42.4	-	PG, DPG, three PL, two GL	MK-7, MK-6, MK-5	anteiso-$C_{15:0}$, iso-$C_{15:0}$	Rod	5–7.5	7.5	35	IBRC-M 10446^T
Piscibacillus halophilus HS224^T	98.5%: *Piscibacillus salipiscarius*	37.5	30.8%	PG, DPG	MK-7	anteiso-$C_{15:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$	Rod	10	7.5	35	DSM 21633^T
Thalassobacillus cyri HS286^T	99.4%: *Thalassobacillus devorans* G-19.1^T	43.0	27.3%	DPG, PG, two PL, a GL	MK-7	anteiso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{14:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$	Rod	8.0	7.5	40	CCM 7597^T
Bacillus persicus B48^T	97.4%: *Bacillus foraminis* CV53^T	40.1	8.1%	DPG, PG, PE, an APL, two unknown PL	MK-7	iso-$C_{15:0}$, anteiso-$C_{15:0}$	Rod	2.5	7.5–8.0	35	IBRC-M 10115^T

Bacillus halosaccharovorans E33^T	99.2%: *Bacillus niabensis* 4T19^T	42.6	22%	DPG, PG, two unknown GL, an unknown L, an unknown PL	MK-7, MK-6, MK-8	anteiso-$C_{15:0}$, iso-$C_{15:0}$	Rod	5–15	7.5–8.0	40	IBRC-M 10095^T
Bacillus salsus A24^T	97.2 %: *Bacillus alkalitelluris* BA288^T	35.9	8%	PG, DPG, PE, two unknown PL	MK-7, MK-6	anteiso-$C_{15:0}$, iso-$C_{15:0}$	Rod	3	8.0	35	IBRC-M 10078^T
Halobacillus karajensis MA-2^T	99.3%: *Halobacillus trueperi* DSM 10404^T	41·3	32.4%	-	MK-7	anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{14:0}$	Rod	10	7.5-8.5	34–38	DSM 14948^T
Salinicoccus iranensis QW6^T	96.4%: *Salinicoccus roseus* DSM 5351^T	54.4	40.3%	-	MK-6	iso-$C_{15:0}$, anteiso-$C_{15:0}$	Cocci	7.5–10.0	7.5	35	DSM 18903^T
Cellulomonas persica I^T	97.2%: *Cellulomonas avigena*	-	62%	-	-	-	Straight or slightly curved rods	-	7.2-7.4	30	ATCC 700642^T
Cellulomonas iranensis O^T	98.4%: *Cellulomonas gelida*	-	39%	-	-	-	Straight or slightly curved rods	-	7.2-7.4	30	ATCC 700643^T
Streptomyces iranensis HM 35^T	99.2%: *Streptomyces rapamycinicus* NRRL 5491^T	71.1	72.7%	PE, hydroxy-PE, PI mannosides, lyso-PE, hydroxy-lyso-PE	MK-9(H_4), MK-9(H_6), MK-9(H_8)	iso- and anteiso-branched components	Filamentous	Grows well in 0–2.5	6-9	28	DSM 41954^T
Nocardiopsis sinuspersici HM6^T	99.2%: *Nocardiopsis quinghaiensis*	71.6	39.6%	PC, PE	MK-10(H_0), MK-10(H_2), MK-9(H_0)	iso-$C_{16:0}$, anteiso-$C_{17:0}$, 10-methyl-$C_{18:0}$, 10-methyl- $C_{17:0}$	Filamentous	2.5	7	28	UTMC 00102^T
Nocardiopsis arvandica HM7^T	99.8%: Nocardiopsis sinuspersici DSM 45277^T	71.5	37.6%	PC, PE, PI, PG, DPG	MK-10(H2), MK-10(H4), MK-10(H0), MK-9(H2)	iso-C16 : 0, anteiso-C17 : 0	Filamentous	Good growth in 0–15	Grows at 6–11	25-30	UTMC 00103^T
Mycobacterium iranicum M05^T	*Mycobacterium gilvum* (MLSA results)	-	-	-	-	iso-C14 : 0, C17 : 1v7c, iso-C20 : 0	Rod	growth in 5	-	37	DSM 45541^T

Abbreviations: Diphosphatidylglycerol:DPG, Phosphatidylglycerol:PG, Aminolipid:AL, Aminophospholipid:APL, Phospholipid:PL, Aminoglycolipid:AGL, Glycolipid:GL, Phosphatidylethanolamine:PE, Aminophospholipid:APL, Lipid:L, Phosphatidylinositol:PI, Phosphatidylcholine:PC

not be a member of the genus *Bacillus*. The DNA G+C content of this new isolate was 38.9 mol%. DNA–DNA hybridization experiments revealed a low level of relatedness between strain P4B^T and *B. persepolensis* HS136^T (6%). The major cellular fatty acids of strain P4B^T were iso-$C_{15:0}$ and anteiso-$C_{15:0}$, as for *B. persepolensis* HS136^T but in contrast to *B. salarius* DSM 16461^T and *B. subtilis* subsp. *subtilis* DSM 10^T. Its polar lipid pattern consisted of phosphatidylglycerol, an aminoglycolipid and an unknown phospholipid. This polar lipid profile was similar to that obtained for *B. persepolensis* DSM 21632^T but different from those of *B. salarius* DSM 16461^T and *B. subtilis* subsp. *subtilis* DSM 10^T. The isoprenoid quinones were MK-7 (88%) and MK-8 (2%). The peptidoglycan contained *meso*-diaminopimelic acid as the diagnostic diamino acid. All these features indicate placement of strain P4B^T within the *Firmicutes*, closely related to *B. persepolensis* but with features clearly distinct from those of the genus *Bacillus* and other related genera. On the basis of these data, strain P4B^T is considered to represent a novel species of a new genus, for which the name *Alteribacillus bidgolensis* gen. nov., sp. nov. is proposed. The type strain of *Alteribacillus bidgolensis* is P4B^T with IBRC-M 10614^T accession number. It is also suggested to transfer *B. persepolensis* to this new genus, as *Alteribacillus persepolensis* comb. nov. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P4B^T is HQ433453 (48).

Alteribacillus persepolensis sp. nov. comb. nov. protologue

Strain HS136^T a Gram-positive and moderately halophilic bacterium was isolated from the hypersaline lake Howz-Soltan in Iran. Cells were motile rods, producing ellipsoidal endospores at a central–subterminal position in non-swollen sporangia. On the basis of 16S rRNA gene sequence analysis, strain HS136^T was shown to belong to the genus *Bacillus* within the phylum *Firmicutes*. The DNA G+C content of this new isolate was 37.1 mol%. The major cellular fatty acids of strain HS136^T were iso-$C_{15:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0,}$ and its polar lipid pattern consisted of phosphatidylglycerol and diphosphatidylglycerol. The isoprenoid quinone was MK-7. The peptidoglycan type is A1γ, with meso-diaminopimelic acid as the diagnostic diamino acid. On the basis of polyphasic evidence from this study, *Bacillus persepolensis* sp. nov. is proposed, with strain HS136^T (=CCM 7595^T=DSM 21632^T) as the type strain. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HS136^T is FM244839 (Amoozegar et al., 2009). This species have been moved to the *Alteribacillus* genus as a new combination *Alteribacillus persepolensis* HS136^T after announcement of this genus (48,49)

Ornithinibacillus halophilus sp. nov. protologue

A novel Gram-stain-positive, moderately halophilic bacterium, designated strain G8B^T, was isolated from water of the hypersaline lake Aran-Bidgol in Iran and characterized taxonomically using a polyphasic approach. Cells of strain G8B^T were rod-shaped, motile and produced oval endospores at a terminal position in swollen sporangia. Strain G8B^T was strictly aerobic, catalase-positive and oxidase-negative. The strain was able to grow at NaCl concentrations of 0.5–12.5% (w/v), with optimum growth occurring at 5–7.5% (w/v) NaCl. The optimum temperature and pH for growth were 35–40 °C and pH 7.5–8.0, respectively. On the basis of 16S rRNA gene sequence analysis, strain G8B^T was

shown to belong to the genus *Ornithinibacillus* within the phylum *Firmicutes*. The DNA G+C content of strain G8B^T was 36.9 mol%. The major cellular fatty acids of strain G8B^T were anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$, and its polar lipid pattern consisted of phosphatidylglycerol, diphosphatidylglycerol, four unknown phospholipids and an unknown aminolipid. The isoprenoid quinones were MK-7 (98%) and MK-8 (2%). Strain G8B^T contained a peptidoglycan of type A4β, l-Orn–d-Asp. All these features confirmed the placement of isolate G8B^T within the genus *Ornithinibacillus*. DNA–DNA hybridization experiments revealed a low level of relatedness (6%) between strain G8B^T and closely related strain. On the basis of evidence from this study, a novel species of the genus *Ornithinibacillus*, *Ornithinibacillus halophilu*s sp. nov., is proposed, with strain G8B^T with accession number IBRC-M 10683^T as the type strain. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain G8B^T is HQ433440 (50).

Lentibacillus persicus sp. nov. protologue

A Gram-staining-positive, moderately halophilic bacterium, designated strain Amb31^T, was isolated from water of the hypersaline lake Aran-Bidgol and characterized taxonomically using a polyphasic approach. Cells were rods, motile and able to produce ellipsoidal endospore at a central position in swollen sporangia. Strain Amb31^T was facultatively anaerobic and catalase- and oxidase-positive. The strain grew in a complex medium supplemented with 3–25% (w/v) NaCl (optimum 7.5–10%). Optimal growth was at 30–35 °C and pH 7.5. Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that strain Amb31^T belonged to the genus *Lentibacillus*. The cell-wall peptidoglycan of strain Amb31^T was based on *meso*-diaminopimelic acid and MK-7 was the respiratory isoprenoid quinone. The major fatty acids were anteiso-$C_{15:0}$ (44.7%), iso-$C_{16:0}$ (21.4%) and anteiso-$C_{17:0}$ (15.9%) and the polar lipid pattern consisted of phosphatidylglycerol, diphosphatidylglycerol, five phospholipids and a glycolipid. The DNA G+C content was 44.1 mol%. All these features confirmed the placement of strain Amb31^T within the genus *Lentibacillus* and the strain could be clearly differentiated from strains of the other species of *Lentibacillus* on the basis of several phenotypic, genotypic and chemotaxonomic features. DNA–DNA relatedness with the type strain of the most closely related strain was 28%. Therefore, strain Amb31^T has been announced as type strain of *Lentibacillus persicus* sp. Nov with accession number of CCM 7683^T. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Amb31^T is FN376846 (51).

Bacillus iranensis sp. nov. protologue

A Gram-positive, moderately halophilic rod, designated X5B^T, was isolated from saline mud of the hypersaline lake Aran-Bidgol. Strain X5B^T was a strictly aerobic, motile bacterium that produced ellipsoidal endospore at a central-subterminal position in non-swollen sporangia. On the basis of 16S rRNA gene sequences, strain X5B^T belonged to the genus *Bacillus*. The DNA G+C content was 42.4 mol%. The major cellular fatty acids were anteiso-$C_{15:0}$ and iso-$C_{15:0}$ and the polar lipid profile consisted of phosphatidylglycerol, diphosphatidylglycerol, three phospholipids and two glycolipids. The diamino acid found in the cell-wall peptidoglycan was *meso*-diaminopimelic acid and the isoprenoid quinones were MK-7 (92%), MK-6 (6%) and MK-5 (2%). On the basis of phylogenetic, chemotaxonomic and phenotypic data, a novel

species of the genus *Bacillus* is proposed, with the name *Bacillus iranensis* sp. nov. The type strain is X5B^T with accession number of IBRC 10446^T at the Iranian biological resource center. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain X5B^T is HQ433452 (52).

Piscibacillus halophilus sp. nov. protologue

Strain HS224^T a Gram-positive, moderately halophilic bacterium was isolated from the hypersaline lake Howz-Soltan. Cells of strain HS224^T were rod-shaped, motile and produced oval endospore. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain HS224^T was affiliated to the genus *Piscibacillus*. Strain HS224^T was able to grow at NaCl concentrations of 1–20% (w/v), with optimum growth occurring at 10% (w/v) NaCl. The optimum temperature and pH for growth were 35 °C and pH 7.5. Major polar lipids were phosphatidylglycerol and diphosphatidylglycerol, the isoprenoid quinone was MK-7 and the peptidoglycan type was A1γ, with *meso*-diaminopimelic acid as the diagnostic diamino acid; these characteristics were shared with the closely related species in the *Piscibacillus* genus. The major cellular fatty acids of strain HS224^T were anteiso-$C_{15:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$. The G+C content of the DNA was 37.5 mol%. The level of DNA–DNA relatedness between strain HS224^T and the closely related species was 30.8%. It is evident from the genotypic, chemotaxonomic and phenotypic data presented that strain HS224^T represents a novel species of the genus *Piscibacillus*, for which the name *Piscibacillus halophilus* sp. nov. is proposed. The type strain is HS224^T with accession numbers of CCM 7596^T and DSM 21633^T. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HS224^T is FM864227 (53).

Thalassobacillus cyri sp. nov. protologue

A Gram-positive, moderately halophilic bacterium, designated strain HS286^T, was isolated from water of the Howz-Soltan hypersaline Lake. Cells were strictly aerobic, rod-shaped, motile and able to produce ellipsoidal endospore at a central-subterminal position in swollen sporangia. Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that strain HS286^T was closely related to *Thalassobacillus devorans* G-19.1^T (99.4% gene sequence similarity). Strain HS286^T had cell-wall peptidoglycan based on *meso*-diaminopimelic acid and MK-7 as the respiratory isoprenoid quinone. The major fatty acids were anteiso-$C_{15:0}$ (43.8%), iso-$C_{16:0}$ (21.4%), iso-$C_{14:0}$ (9.4%), anteiso-$C_{17:0}$ (8.7%) and iso-$C_{15:0}$ (7.0%) and the polar lipid pattern consisted of diphosphatidylglycerol, phosphatidylglycerol, two phospholipids and a glycolipid. The DNA G+C content was 43.0 mol%. All of these features confirmed the placement of isolate HS286^T within the genus *Thalassobacillus*. However DNA–DNA hybridization between strain HS286^T and the only recognized species of the genus *Thalassobacillus, T. devorans* G-19.1^T, was 27.3%. on the basis of several phenotypic, genotypic and chemotaxonomic features strain HS286^T constitutes a novel species, for which the name *Thalassobacillus cyri* sp. nov. is proposed. The type strain is HS286^T with CCM 7597^T and JCM 15722^T accession numbers. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HS286^T is FM864226 (54).

Bacillus persicus sp. nov. protologue

Strain B48^T a novel Gram-positive and slightly

halophilic bacterium was isolated from soil around the hypersaline lake Aran-Bidgol. Cells of strain B48^T were strictly aerobic, catalase- and oxidase-positive, non-motile rods and produced ellipsoidal endospores at a central or subterminal position in swollen sporangia. The strain was able to grow at NaCl concentrations of 0.5–10.0% (w/v), with optimum growth occurring at 2.5% (w/v) NaCl. The optimum temperature and pH for growth were 35 °C and pH 7.5–8.0, respectively. On the basis of 16S rRNA gene sequence analysis, strain B48^T was shown to belong to the genus *Bacillus* within the phylum *Firmicutes*. The DNA G+C content of this new isolate was 40.1 mol%. The major cellular fatty acids of strain B48^T were iso-$C_{15:0}$ and anteiso-$C_{15:0,}$ and its polar lipid pattern consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an aminophospholipid and two unknown phospholipids. The only quinone present was menaquinone 7 (MK-7). The peptidoglycan contained *meso*-diaminopimelic acid as the diagnostic diamino acid. All these features confirm the placement of isolate B48^T within the genus *Bacillus*. DNA–DNA hybridization experiments showed 8.1% relatedness between strain B48^T and the closely related species. On the basis of polyphasic evidence from the study, a new species of the genus *Bacillus*, *Bacillus persicus* sp. nov., is proposed, with strain B48^T (IBRC-M 10115^T) as the type strain. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B48^T is HQ433471 (55).

Bacillus halosaccharovorans sp. nov. protologue

A novel Gram-stain-positive, moderately halophilic bacterium was isolated from water of the hypersaline lake Aran-Bidgol and characterized taxonomically using a polyphasic approach. Cells of strain E33^T were motile rods and produced ellipsoidal endospores at a central or subterminal position in swollen sporangia. Strain E33^T was a strictly aerobic bacterium, catalase- and oxidase-positive. On the basis of 16S rRNA gene sequence analysis, strain E33^T was shown to belong to the genus *Bacillus* within the phylum *Firmicutes* . The DNA G+C content of the type strain of the novel species was 42.6 mol%. The major cellular fatty acids of strain E33^T were anteiso-$C_{15:0}$ and iso-$C_{15:0,}$ and the polar lipid pattern consisted of diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids, an unknown lipid and an unknown phospholipid. The isoprenoid quinones were MK-7 (97%), MK-6 (2%) and MK-8 (0.5%). The peptidoglycan contained *meso*-diaminopimelic acid as the diagnostic diamino acid. All these features confirm the placement of isolate E33^T within the genus *Bacillus*. DNA–DNA hybridization experiments revealed low levels of relatedness between strain E33^T and closely related species were less than 70% which means this strain belongs to a new species. On the basis of polyphasic evidence from this study, a novel species of the genus *Bacillus*, *Bacillus halosaccharovorans* sp. nov. is proposed, with strain E33^T with IBRC-M 10095^T accession number as the type strain. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Bacillus halosaccharovorans* E33^T is HQ433447 (56).

Bacillus salsus sp. nov. protologue

A Gram-staining-positive and slightly halophilic bacterium, designated strain A24^T, was isolated from the hypersaline lake Aran-Bidgol in Iran. Cells of strain A24^T were rod-shaped, strictly aerobic, motile rods and produced oval endospore at a terminal position in swollen sporangia. Strain A24^T was catalase- and oxidase-positive. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain

A24^T belonged to the genus *Bacillus* within the phylum *Firmicutes*. The G+C content of the genomic DNA of this strain was 35.9 mol%. The polar lipid pattern of strain A24^T consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and two unknown phospholipids. The major cellular fatty acids of strain A24^T were anteiso-$C_{15:0}$ and iso-$C_{15:0}$. The respiratory quinones were MK-7 (94 %) and MK-6 (4 %). The peptidoglycan contained meso-diaminopimelic acid as the diagnostic diamino acid. All these features confirm the placement of isolate A24^T within the genus *Bacillus*. DNA-DNA hybridization experiments revealed a relatedness of 8% between strain A24^T and closely related species supporting its placement as a new species. On the basis of Phenotypic characteristics, phylogenetic analysis and DNA-DNA relatedness data this strain represents a novel species of the genus *Bacillus*, for which the name *Bacillus salsus* sp. nov. is proposed. The type strain is strain A24^T (IBRC-M 10078^T) (57).

Halobacillus karajensis sp. nov. protologue

A moderately halophilic, Gram-positive, spore-forming bacterium was isolated from surface saline soil of the Karaj region, Iran. Strain MA-2^T, was strictly aerobic with rod-shaped cells that occurred singly, in pairs or short chains. It contained l-Orn–d-Asp-type peptidoglycan and the major respiratory lipoquinone was MK-7. It was non-motile and had an ellipsoidal endospore located centrally or subterminally. The DNA G+C content was 41.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain MA-2^T was associated with *Bacillus* rRNA group 1. On the basis of phenotypic and chemotaxonomic characteristics, 16S rRNA gene sequence analysis and DNA–DNA hybridization data, it is proposed that strain MA-2^T (=DSM 14948^T =LMG 21515^T) should be placed in the genus *Halobacillus* as the type strain of a novel species, *Halobacillus karajensis* sp. nov. The EMBL accession number for the 16S rDNA sequence of strain MA-2^T (=DSM 14948^T) is AJ486874 (58).

Salinicoccus iranensis sp. nov. protologue

A novel moderately halophilic, Gram-positive bacterium, designated strain QW6^T, was isolated from textile industry wastewater in Qom, Iran. Strain QW6^T was strictly aerobic, non-motile, non-sporulating and oxidase- and catalase-positive. Phylogenetic analysis based on 16S rRNA gene sequence similarity indicated that strain QW6^T is a member of the genus *Salinicoccus*. The organism possessed Lys- and Gly-containing peptidoglycan. Strain QW6^T had iso-$C_{15:0}$ and anteiso-$C_{15:0}$ as the predominant fatty acids and MK-6 as the major respiratory lipoquinone. The chemotaxonomic profile of this organism was consistent with its classification in the genus *Salinicoccus*. The DNA G+C content of strain QW6^T was 54.4 mol%. On the basis of phenotypic characteristics, 16S rRNA gene sequence analysis and DNA–DNA hybridization of less than 50% to species of the genus *Salinicoccus*, it is proposed that strain QW6^T (=DSM 18903^T=CCM 7448^T) should be placed in the genus *Salinicoccus* as the type strain of a novel species, *Salinicoccus iranensis* sp. nov. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain QW6^T is DQ767692 (59).

Cellulomonas persica sp. nov. and *Cellulomonas iranensis* sp. nov. protologue

Two newly described species of mesophilic, cellulose-degrading, aerobic bacteria were

isolated from forest humus soils along the southern border of the Caspian Sea. *Cellulomonas persica* and *Cellulomonas iranensis* are proposed as new specific epithets based on comparative sequence analyses of 16S rDNA, DNA-DNA hybridization and phenotypic characteristics. Strain I^T as type strain *Cellulomonas persica* sp. nov has the accession number of ATCC 700642^T and strain O^T has the accession number of ATCC 700643^T as type strain of *Cellulomonas iranensis* sp. nov. (60).

Streptomyces iranensis sp. nov. protologue

Strain HM 35^T was isolated from soil in Isfahan city, Iran. Strain HM 35^T produced a branched substrate mycelium and aerial hyphae that developed into short, compact, spiral spore chains with grey rugose spores at the tips of the aerial hyphae. On some media, these spirals coalesced into dark masses of spores with age. Whole-cell hydrolysates of strain HM 35^T contained ll-diaminopimelic acid, glucose and ribose. Phospholipids profile of the cell included phosphatidylglycerol, hydroxy-phosphatidylethanolamine, lyso-phosphatidylethanolamine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylinositol mannosides and hydroxy-lyso-phosphatidylethanolamine. MK-9(H_4), MK-9(H_6) and MK-9(H_8) were the predominant menaquinones. The major fatty acids included iso- and anteiso-branched components. The chemotaxonomic characteristics of the novel isolate matched those described for members of the genus *Streptomyces*. Based on 16S rRNA gene sequence similarity analysis strain HM 35^T belongs to the *Streptomyces* genus and it formed a distinct monophyletic line within the 16S rRNA gene sequence tree. The level of DNA–DNA relatedness between strain HM 35^T and the type strain of *S. rapamycinicus* was 72.7%. Strain HM 35T showed the typical morphology found among members of the *S. violaceusniger/ Streptomyces hygroscopicus* group but could be clearly differentiated from closely related species based on other phenotypic markers. On the basis of phenotypic and genotypic data strain HM 35^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces iranensis* is proposed. The type strain is HM 35^T with DSM 41954^T and CCUG 57623^T accession numbers. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HM 35^T is FJ472862 (61).

Nocardiopsis sinuspersici sp. nov. protologue

Strain $HM6^T$ a halotolerant bacterium isolated from sandy rhizospheric soil in Sarbandar, Persian Gulf, Iran. According to polyphasic approach of bacterial systematic strain $HM6^T$ represents a novel species within the genus *Nocardiopsis*. 16S rRNA gene sequence similarity analysis revealed that strain $HM6^T$ clustered with strains of the genus *Nocardiopsis* and it showed DNA–DNA relatedness less than 39.6%. On the basis of genotypic and phenotypic differences between strain $HM6^T$ and closely related species of the genus *Nocardiopsis* a novel species, *Nocardiopsis sinuspersici* sp. nov., is proposed. The type strain is $HM6^T$ with UTMC 00102^T, DSM 45277^T and CCUG 57624^T accession numbers. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain $HM6^T$ is EU410476 (62).

Nocardiopsis arvandica sp. nov. protologue

Strain $HM7^T$ was isolated from sandy soil from the banks of the Arvand River, Khoramshahr, Iran. Strain $HM7^T$ had long, branched substrate mycelia and the aerial mycelium completely fragmented to long chains of rod-shaped spores. The cell wall of strain $HM7^T$ contained

meso-diaminopimelic acid, galactose and ribose. The predominant phospholipids were phosphatidylglycerol, phosphatidylethanolamine diphosphatidylglycerol,and phosphatidylcholine. The main menaquinones were MK-10(H_2), MK-10(H_4), MK-10(H_0) and MK-9(H_2). Phylogenetic analysis based on 16S rRNA gene sequence similarity suggested that the strain HM7^T is a member of genus *Nocardiopsis* and it showed low DNA–DNA relatedness values with the closely related species. On the basis of Physiological, biochemical and phenotypic differences strain HM7^T is considered as a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis arvandica* sp. nov. is proposed; the type strain of this species is HM7^T with UTMC 00103^T, DSM 45278^T and CCUG 58831^T accession numbers. The GenBank/ EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UTMC 00103^T is EU410477 (63).

Mycobacterium iranicum sp. nov. protologue

Eight independent strains were isolated from clinical specimens from six different countries of the world, two of them were isolated in Iran from cerebrospinal fluid. DNA-based analysis revealed unique sequences in the four regions investigated: the 16S rRNA gene, the rRNA gene internal transcribed spacer 1 and the genes encoding the 65 kDa heat-shock protein and the beta-subunit of RNA polymerase. The phylogenetic analysis placed the strains among the rapidly growing mycobacteria. The genotypic and phenotypic data both strongly supported the inclusion of the strains investigated here as members of a novel species within the genus *Mycobacterium*; the name *Mycobacterium iranicum* sp. nov. is proposed. The type strain is M05^T with accession numbers of DSM 45541^T, CCUG 62053^T and JCM 17461^T. The GenBank/EMBL/ DDBJ accession numbers for the 16S rRNA gene sequence of strain M05^T is HQ009482; for the rpoB gene sequence of strain M05^T is HQ009483; for the ITS sequence of strain M05^T is HQ009484 and for the hsp65 gene sequence of strain M05^T is HQ009485 (64).

Kribbella shirazensis sp. nov. protologue

Strain UTMC 693^T was isolated from soil in Shiraz, Fars province, Iran. Strain UTMC 693^T produced an extensively branched substrate mycelium and aerial hyphae, consisting of hyphae that fragment into short to elongated rod-like elements. Phylogenetic analysis based on 16S rRNA similarity analysis suggested that Strain UTMC 693^T is a member of genus *Kribbella* and DNA-DNA hybridization studies showed low values of relatedness with closely related species. On the basis of genotypic, chemotaxonomic and phenotypic differences between this strain and closely related species strain UTMC 693^T is considered the type strain of a novel species in the genus *Kribbella* with accession numbers of DSM 45490^T and CCUG 61792^T , for which the name *Kribbella shirazensis* is proposed (65).

Conclusion

A biodiversity hotspot is a region with a high level of endemic species that is under threat from humans (66); some regions of Iran are biodiversity hotspots which makes them an important target for new microbial taxa isolation and classification. Despite this fact that we have a rich reservoir of the microbial world in Iran the number of new taxa that have been introduced internationally is not satisfactory. The microbial flora of Iran deserves urgent attention. It is hoped that both researchers and supporting organizations will address this issue.

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