PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
Ministry of Higher Education and Scientific Research
University of Ghardaïa

Faculty of Natural, Life and Earth Sciences

Department of Biology

Thesis submitted in partial fulfilment of the requirements for

Academic Master Degree

In : Biological Sciences

Specialty: Applied Microbiology

Submitted by : EL ALOUANI Daoud
KOUZRITE Mustafa

Molecular and chemotaxonomic comparative study (based on *Jaccard*'s and *Kulczynski*'s coefficients) of the genus *Saccharomonospora*

Presented publicly in: 13 / 06 /2023

Board of examiners :

M. DJELLID Youssef	M. conférence B	Univ. of Ghardaïa	President
M. BOURAS Noureddine	Professor	Univ. of Ghardaïa	Supervisor
M DJEMOUAI Nadjette	M. conférence A	Univ. of Ghardaïa	Examiner

Academic year : 2022/2023

AKNOWLEDGMENTS

We begin by thanking Allah, who bestowed upon us the courage and perseverance to complete this humble work.

We express our deepest gratitude to Professor BOURAS Noureddine from the University of Ghardaïa, for agreeing to supervise this thesis, and for his invaluable assistance, insightful guidance, enriching observations, numerous advices, and support throughout this project.

We would also like to extend our sincere appreciation to Dr DJELLID Youssef, and Dr DJEMOUAI Nadjette for reviewing this work and for their valuable comments and remarks.

Finally, we express our gratitude to the university of Ghardaïa, and to all of those who, directly or indirectly, contributed to the realization of this thesis.

ملخص

تم إجراء دراسة كيميائية (على أساس معاملي *Jaccard* و *Kulczynski*) الإحصائيين، وكذلك دراسة جزيئية على خمسة عشر نوعا (ونوعا فرعيا) من البكتيريا الهيفية التي تنتمي إلى جنس *Saccharomonospora*.

يهدف العمل الحالي إلى إنشاء مقارنة كيميائية وجزيئية بين الأنواع المحددة، من خلال تحديد أقرب الأنواع إلى *S. piscinae* وفقًا لدرجة التشابه، والمسافة التطورية.

تم إجراء تحليل بيانات التركيب الكيميائي الخلوي للأنواع قيد الدراسة، والتي تم تجميعها من المادة العلمية المتاحة لتحديد هذه الأنواع وتصنيفها، وذلك باستخدام مؤشرين إحصائيين: *Jaccard* و *Kulczynski* ؛ وتمت مقارنتها بنتائج التقارب الجزيئي التي تم الحصول عليها باستخدام قواعد البيانات الجزيئية المتخصصة في تسلسلات القواعد النكليوتيدية لشفرة الرنا الريبوزي S16، من أجل إبراز أوجه التشابه بين الأنواع من جهة، ومقارنة ترتيب درجة التقارب بينها وبين النوع *S. piscinae* من خلال كلا الطريقتين.

بينت المقارنة أن الدراسة الكيميائية من خلال معامل *Jaccard* والدراسة الجزيئية أعطتا نفس ترتيب التقارب، الذي هو مختلف جزئيا عن نتيجة الفحص الكيميائي بمعامل *Kulczynski*. في حين أعطى الجميع بنية شجرة أنساب متقاربة عموما.

نستخلص من الدراسة أنه بالرغم من الحصول على ترتيب تقارب متطابق بين الطريقة الجزيئية والطريقة الكيميائية بمعامل *Jaccard*، إلا أنه يستحسن أخذ نتائج الدراسة الكيميائية عموما بتحفظ لما فيها من نقائص ولكون الدراسة الجزيئية أفضل في الفصل بين الأنواع داخل الجنس الواحد، ومع الاستعانة بفحص الجينوم الكلي وغير ذلك في حالات الالتباس أو التقارب الشديد.

الكلمات المفتاحية: *Saccharomonospora* ، دراسة تصنيفية كيميائية، معامل *Jaccard*، معامل *Kulczynski*، التشابه، *S. piscinae*.

Résumé

Une étude chimiotaxonomique (à la base des coefficients de *Jaccard* et *Kuczynski*) et moléculaire a été menée sur quinze espèces et sous-espèce publiées et validées, d'actinobactéries appartenant au genre *Saccharomonospora*.

Le présent travail a pour objectif d'établir une comparaison chimiotaxonomique et moléculaire entre les espèces désignées en déterminant les espèces les plus proches par rapport à *S. piscinae* (utilisée comme référence), et le degré de similarité selon les différent méthodes.

L'analyse chimiotaxonomique a été effectuée à l'aide de deux coefficients statistiques : *Jaccard* et *Kuczynski*, en exploitant les données obtenues à partir de la littérature scientifique de la composition cellulaire durant l'identification et la classification des espèces de l'étude. L'étude moléculaire, est basée sur des algorithmes bioinformatiques capables d'aligner des séquences nucléotidiques du gène 16S ARNr, afin de mettre en évidence les similitudes et les distances entre les espèces et sous-espèces.

Les résultats montrent une consistance entre la chimiotaxonomie basée sur l'indice de *Jaccard* avec celle de l'étude moléculaire. Tandis que l'ordre de similarité et différent entre les deux indices statistique, même qu'il montre une topologie des dendogrammes globalement similaire.

Le genre *Saccharomonospora* a prouvé difficile à classiffier au niveau d'espèce par les charactéristique chimiques seules, et les approches moleculaires sont necessaires et plus conclusives.

Mots clés : *Saccharomonospora*, chimiotaxonomie, coefficient de *Jaccard*, coefficient de *Kulczynski*, similarité, *S. piscinae*.

Abstract

A comparative study between the chemotaxonomy (based on *Jaccard* and *Kulczynski* coefficients') and molecular phylogeny based on the 16S rRNA gene sequences, of 15 species and subspecies of the genus *Saccharomonospora*, was carried out.

The objective of this study is to deduce the evolutionary distances between the studied species and subspecies in reference to *Saccharomonospora piscinae* by the chemotaxonomic and molecular approaches, and work out the consistency between the 2 approaches.

Chemical analysis results(mainly cellar wall components) were retrieved from the literature, analysed statistically using *Jaccard*'s and *Kulczynski*'s coefficients, and similairy orders were established accordingly. In addition, dendograms were constructed based on the Neighbour Joining method using *Past4.12b* software. The phylogenetic distances were obtained from the phylogetic tree based on the 16S rRNA gene sequences using MEGA11 software, based on the Neighbour Joining method.

The results showed a consistanty between the molecular approach using Neighbour Joining method and the chemotaxonomy based on the coefficient of *Jaccard*, while both coefficients exhibited an overall similar dendogram topology, eventhough the similarity order was not completly the same.

In conclusion, due to the descripencies in the chemotaxonomic study, the results should be taken reservedly, and more statistical coeffiecients should be investigated in the future. The molecular study based on 16S rRNA and whole genom approache still offer far better tool for the taxonomy of *Saccharomonospora* at the species and subspecies levels.

Key words: *Saccharomonospora*, chemotaxonomy, coefficient of *Jaccard*, coefficient of *Kulczynski*, similarity, *S. piscinae.*

SUMMARY

Table of contents

List of abbreviations

AM : Aerial Mycelium

BGCs : Biosynthetic Gene Clusters

DAP : Diaminopimelic acid

dDDH : Digital DNA-DNA Hybridation

DDH : DNA-DNA Hybridation

DNA : Deoxyribonucleic acid

FASTA : FAST-All

G+C : Guanine + Cytosine

HAC : Hierarchical Ascendant Classification

IJSEM : International Journal of Systematic and Evolutionary Microbiology

ISP: International *Streptomyces* Project

LPSN : List of Prokaryotic names with Standing in Nomenclature

MEGA : Molecular Evolutionary Genetics Analysis

MK : Menaquinone

MR : Methyl Red

NJ: Neighbour Joining

NRPS : Non-ribosomal peptide synthetase

PAST : PAleontological STatistics

PCR : Polymerase Chain Reaction

PGPR : Plant growth promoting rhizobacteria

PKS : Polyketide synthase

rRNA : Ribosomal ribonucleic acid

SM : Substrate Mycelium

VP : Voges-Proskauer

WGS : Whole Genome Sequencing

Wt/Vol: Weight to volume

List of tables

List of figures

INTRODUCTION

Introduction

Actinobacteria is a highly diverse group of prokaryotes that makes up one of the largest and diverse phylum of bacteria, characterised with high guanine-cytosine content (above 55%). Moreover; actinobacteria exhibit an extremely wide morphological diversity, ranging from cocci to perfect mycelial forms (Goodfellow, 2012). Although most actinobacteria are chemoorganotrophic, mesophilic, neutrophilic, non-halophilic and non-nitrogen-fixers, there is nevertheless a surprising physiological diversity as thermophiles, psychrophiles, alkalophiles, acidophiles, halophiles and nitrogen fixers (Goodfellow *et al.*, 2012). This great metabolic diversity gives advantage to actinobacteria to colonise practically all the habitats, including the most extreme ecological niches where life was considered to be impossible (Tiwari and Gupta, 2013). Moreover; the capacity of *Actinobacteria* to produce enzymes, primary and secondary bioactive metabolites, made them of an extreme biotechnological interest (Ramírez-Durán *et al.*, 2021).

The taxonomy and classification of *Actinobacteria* relies on a polyphasic approach (Vandamme *et al.*, 1996), which compiles the results of the phenotypic (morphological and physiological), chemotaxonomic (numerical) and genotypic (molecular) methods. Classical phenotypic tests are the basis for the description of the species, up to the family, and takes in considerations all the morphological, physiological, and biochemical features. The fact that the phenotype is the expression of the genotype as a result of the interaction of genes with the cultural conditions should make it a true reflection of the genotype. Therefor; strains from related taxa should be compared to their phenotypic features under identical conditions and protocols. The molecular phylogeny in prokaryotes, is based heavily on comparing the sequences of 16S rRNA gene, the relationships are disclosed as a degree of similarity (%), or on the form of phylogenetic trees where the branches length reflects the degrees of genetic divergence (Li *et al.*, 2016) in reference to *S. piscinae*, that was selected for the reason of being the most recent validated species (Tseng *et al.*, 2018)

In this work, we try to establish the reliability of the numerical (chemotaxonomic) method (based on *Jaccard* and *Kulczynski* coefficients') in comparison with the molecular approach based on the 16S rRNA gene phylogeny, using the Neighbour Joining method. In total, 15 validated species and subspecies of the genus *Saccharomonospora*, has been the subject to this study and the relatedness to *Saccharomonospora piscinae* is worked out by the different approaches.

BIBLIOGRAPHIC REVIEW

CHAPTER I: Bibliographic review

1.1 *Actinobacteria*

1.1.1 An introduction to *Actinobacteria*

Actinobacteria are defined as Gram-positive, fungi-like bacteria, with an elevated G+C content (from 55% to over 70%). The phylum is very diverse phenotypically and morphologically (from cocci to highly differentiated mycelia). Most species are aerobic, facultative anaerobic or anaerobic (Goodfellow *et al.*, 2012).

Actinobacteria are metabolically very diverse, predominantly chemo-organotrophs, and they play a major role in composting organic matters, especially slow degrading bio-materials such as cellulose, chitin, and lignin, and contribute subsequently to the formation of hummus in the soil (Ranjani *et al.*, 2016).

Actinobacteria are distributed in all the ecosystems: in the soil, fresh and marine waters, and even in the extreme habitats (thermophilic, acidophilic, and halophilic environments). They are also involved in beneficial associations with other organisms, such as in the gut microbiota (*Bifidobacterium*), in symbiosis with plants (nitrogen fixators. The most majority of actinobacteria are beneficial, but animal and plant pathogens are not uncommon, to name for example the human tuberculosis causing agent: *Mycobacterium tuberculosis* (Jensen and Lauro, 2008), *Streptomyces scabies* is the causal agent of scab disease to several crops (Ismail *et al.*, 2020), and Actinomyces bovis that causes the bovine actinomycosis (Cunha *et al.*, 2022).

Actinobacteria are also of undisputed economic and industrial value, for their well-recognized capacity of primary and secondary metabolites production. In fact, Subramani and Sipkema (2019) have reported that until 2010, 13700 bioactive molecules have been discovered to be produced by actinobacteria. That includes antibiotics, antitumor, antiparasitic, immunomodulator, plant growth stimulators, phytohormones, and pesticides. Actinobacteria enzymes are exploited in food, pharmaceutical, and chemical industries, as well as in the fields of bioremediation, bioconversion, and nano molecules technology applications. Actinobacteria are also promising biocontrol tools against plant pathogens for their antagonistic activities (Ranjani *et al.*, 2016).

1.1.2 Systematic of *Actinobacteria*

The phylum *Actinobacteria* is one of the largest taxonomic units within the *Bacteria* domain (Ludwig *et al.*, 2012).

The phylum *Actinobacteria* is divided into six classes. The class *Actinobacteria* comprises of 15 orders, 43 families, and 203 genera. All Actinobacteria are included under the order *Actinomycetales*, commonly known as A*ctinomycetes*. The order *Actinomycetales* is in turn divided into four families: *Streptomycetaceae, Actinomycetaceae, Actinoplanaceae*, and *Mycobacteriaceae* (Goodfellow *et al.*, 2012). The molecular identification on the base of 16S rRNA gene sequences is the most significant tool in the classification of actinobacteria (Ranjani *et al.*, 2016).

The genus *Saccharomonospora* was created by Nonomura and Ohara in 1971 (Nonomura and Ohara, 1971), and approved in 1980. The type species is *Saccahromonospora viridis* (Schuurmans *et al.*, 1956). The classification of *S. viridis* is as follow:

Domain: *Bacteria*

Phylum: *Actinobacteria*

Class: *Actinobacteria*

Sub-class: *Actinobacteridae*

Order: *Actinomycetales*

Sub-order: *Pseudonocardineae*

Family : *Pseudonocardiaceae*

Genus : *Saccharomonospora*

Type species : *Saccharomonospora viridis* (Schuurmans *et al.*, 1956)

1.1.3 Taxonomy of *Actinobacteria*

According to Gillis *et al.* (2015) “Bacterial taxonomy comprises the interrelated areas of classification, nomenclature, and identification and is supposed to reflect phylogeny and evolution”.

Early classifications at the genus level, were based on morphological (macro and micromorphology), physiological (cultural and biochemical), and chemotaxonomic characters (Lechevalier and Lechevalier, 1965). Subsequently, new statistical methods are introduced to compute the bulk of physiological and chemical taxonomic data, previously done by hand (Ludwig *et al.*, 2012).

In the late 1950s, the numerical taxonomy also known as phenetics or taximetrics emerged (Vane-Wright, 2013). The development of computers, made it possible to analyse large number of phenotypic traits from a large number of strains, and generated matrices that show the degree of similarity between each pair of strains. Constructed dendograms reveal the general picture of phenotypic characters within a group. Sooner it became evident that large numbers of phenotypic traits are taxonomically relevant, and indeed imitate the genotypic information (Vishaka *et al.*, 2019).

Polyphasic taxonomy, a term coined by Colwell in 1970, is another major development in prokaryotes taxonomy. It is comprehensive approach of all the morphological, physiological, chemotaxonomic, pathogenicity, and molecular methods of microorganisms, and essentially indicates a consensus type of taxonomy and it has been used to delineate taxa at all levels (Vandamme *et al.*, 1996).

With the comming of DNA amplification and sequencing techniques (16S rRNA gene in particular), molecular taxonomy has emerged, and it became crucial in the identification and determination of the taxonomic status (Ludwig and Klenk, 2005). The 16S rRNA gene has proven to be the best molecular tool for the molecular phylogeny of bacteria including actinobacteria, for its omnipresence in all the bacteria, and its highly conserved regions. Some other molecular methods have been in use such as DNA hybridisation, DNA G+C content, and the genomic annotation (Chen *et al.*, 2016). However, the phenotypic features of a new species still difficult to be predicted even with the complete genomic sequence in hand (Krieg and Padgett, 2011).

1.1.3.1 Phenotypic taxonomy

The phenotypic taxonomy relay on morphological, physiological and biochemical traits, and had been the oldest tool for the characterization and classification of prokaryotes. It takes in consideration information on cultural conditions, such as temperatures, pH, salinity, atmospheric conditions, the growth in the presence of antagonist substances, enzymatic activities not necessarily related to the energetic metabolism, and the capacity of degradation and metabolisation of various compounds and carbon sources, pathogenicity and its factors, and so on (Li *et al.*, 2016). The work of Smibert and Krieg (1994), named “Phenotypic characterization” is considered the corner stone of this topic (Tindall *et al.*, 2010).

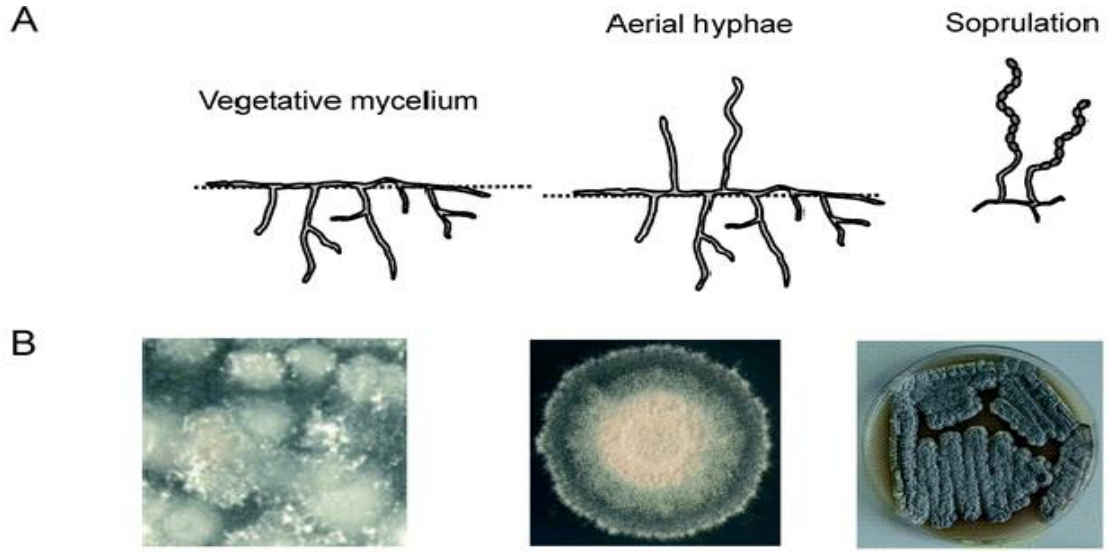
1.1.3.1.1 Morphological identification

The description of the morphology is very important at the genus level. Cultural, macromorphologic and micromorphologic characters are assessed according to « Bergey's Manual » of 1989 and 1994 (Boudjellal, 2012).

1.1.3.1.1.1 Macromorphology

Actinobacteria when grown on agar substrate, form a mycelium, which could be substrate mycelium (SM), aerial mycelium (AM) or both (Figure 1). In one hand, mycelial fragmentation can be considered as a form of vegetative reproduction; in the other hand, mycelial lifestyles usually reproduce asexually by spore formation (Barka *et al.*, 2016).

Figure 1. Steps of *Streptomycetes*' development illustration, drawing (A) correspond to pictures (B) (Worrall and Vijgenboom, 2010).



Cultures (colony) description is very important for differentiation at the supra-genera level (Boudjelal-bencheikh, 2012). The most important features are as follow :

- Production or non-production of AM, and its colour (Figure 2).
- Presence or absence of SM, and its colour.
- Production and colour of melanoid pigments.

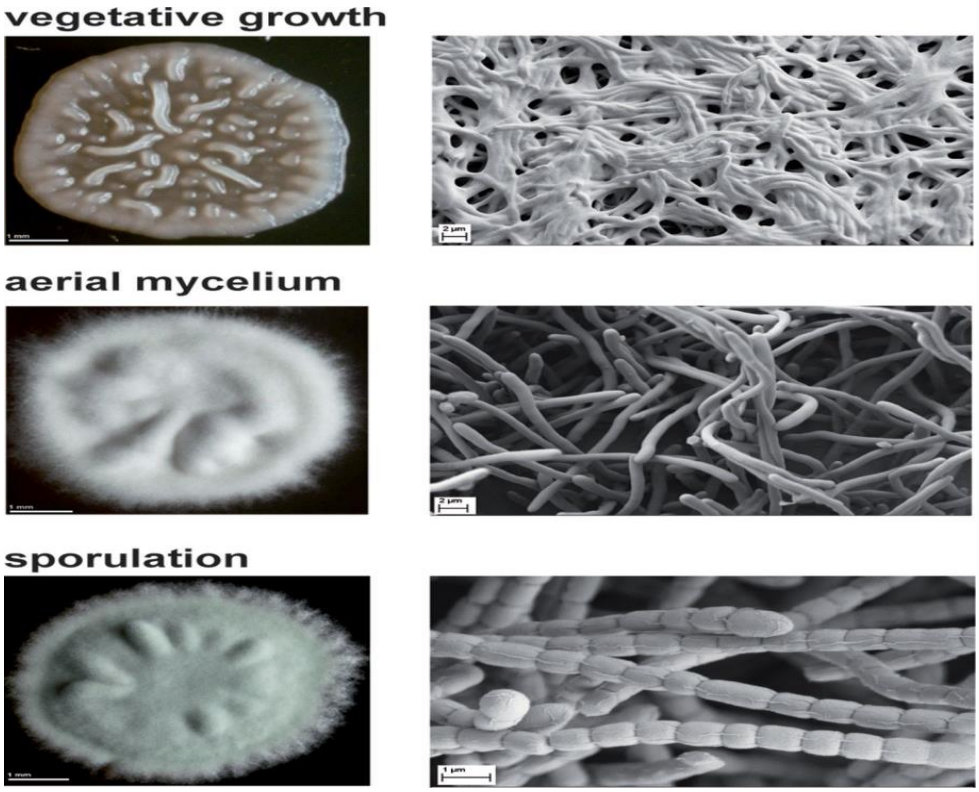


Figure 2. Macroand micromorphology of *Streptomyces venezuelae*.
Left: Colony morphology and colour; right: scanning electron micrographs (Tschowri, 2016).

1.1.3.1.1.2 Micromorphology

The diversity of cell shapes and sizes, and the underlying sub cellular structures, spores and their morphology, can be described by scanning electron microscopes. The infrastructures of the cell and the cytoplasmic inclusions are described with transmission electron micrographs (Tindall *et al.*, 2010). The spores and their morphology are extremely useful in the taxonomy of *Actinobacteria* (Goodfellow and Williams, 1983).

The micrmophological traits elaborated extensively by Li *et al.* (2016), are summarised as follow:

- Fragmentation, or non- fragmentation of the SM.
- Spore formation/non-formation on the S/AM.
- Spore chain length, shape, position, colour, and motility (Figure 3).
- Surface ornamentation of spores (Figure 4).
- Sporangia position, shape, and sporangiospores with or without flagella.

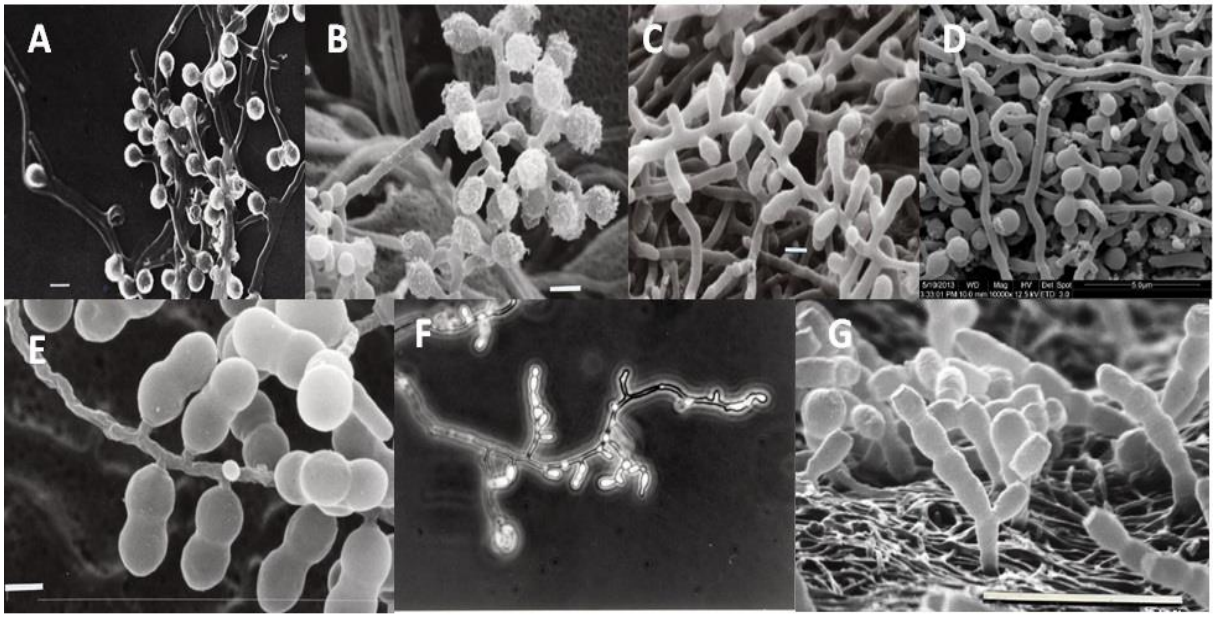


Figure 3. Micrography of spore production and spores in short chains.
(A) *Micromonospora* sp. SF2259^{T} (B) *T. alba* JCM 3077^{T} (C) *S. viridis* IFO 12207^{T} (D) *T. daqus* H-18 (E) *M. rosea* JCM 3006^{T} (F) *N. brevicatena* A444 (G) *Catellatospora* sp. MB-VE 1321. Images taken from Li *et al.* (2016).

Some spore surface ornamentations are illustrated in figure 4.

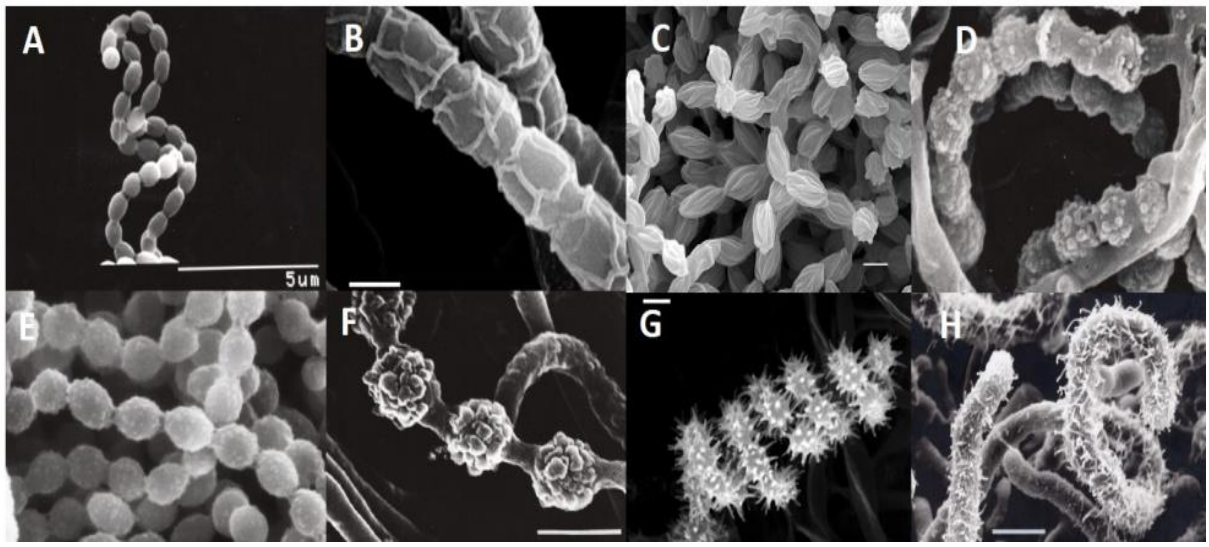


Figure 4. Micrography of spore production in long chain.
(A) Rectiflexible spore chains of *S. actuosus* U 227 (B) Looped (Retinaculiaperti) spore chains of *S. vinaceus* (C) Spiral spore chains of *Streptomyces* sp. SF 2587 (D) Verticillati spore chains of *S. verticillus* AT 291 (E) Fragmenting branched aerial hyphae of *N. lucentensis* IFO 15854T (F) Spore chains, in hooks, curves or spirals of one turn in *A. verrucosospora* JCM 3147T (G) Spiny spores in spirals of *Streptomyces* sp. WK-1875 (H) Hairy spores of *S. finlayi* JCM 4637T . Images taken from Li *et al.* (2016).

1.1.3.1.2 Physiological and biochemical identification

The use of physiological and biochemical traits in the systematics of actinobacteria, in addition to its ease of use, still very meaningful even with the advent of genomics and proteomics. Regulatory proteins and microbial enzymes are products of genes expression, and their comparison is a sort of gene comparison. Physiological and biochemical description is of primary importance at the genus and species level (Li *el al.*, 2016).

The physiological and biochemical traits include information on growth conditions (temperatures, pH, salinity, aerobic/anaerobic atmosphere), antimicrobial resistance, data on the enzymatic capacities, degradation and metabolisation of various compounds, and so on (Li *et al.*, 2016) (Table 1).

To have pertinent results from physiological and biochemical tests, many factors must be taken in consideration. Firstly, the strain should be compared to closely related type strains and other strains according to 16S rRNA analyses. Secondly, as the phenotypic characteristics can be influenced by many factors such as cultural condition and others, a rigorous and preferably standardized methodology must be followed to get comparable results with other previous studies, and the performance of duplicate or triplicate, and the design of reasonable positive and negative controls is of major importance (Xu *et al.*, 2007).

Table 1. Common physiological and biochemical characteristics used for classification and identification of *Actinobacteria* (Li *et al.*, 2016).

Characteristic	**The difference between groups**
Temperature	Optimal, lowest, and highest growth temperature
pH values	The range of pH values of growth, and the optimal growth pH
Osmotic pressure	Salt concentration and halophilism
Utilisation of nitrogen source	Proteins, peptones, amino acids, nitrogen, inorganic salts, *etc*.
Utilisation of carbon sources	Simple and complex saccharides, alcohols, and organic acids, and acid production from carbohydrates
Growth factors	Special vitamins, amino acids, X factor (hemin) and V factor (nicotinamide-adenine-dinucleotide, NAD) requirements
Atmospheric condition	Aerobic, microaerophilic, anaerobic, facultative anaerobic
Antimicrobial activity	Inhibition of Gram + and Gram + bacteria, fungi and yeast

Table 2. –continued- Common physiological and biochemical characteristics used for classification and identification of *Actinobacteria* (Li *et al.*, 2016).

Metabolisation	Characteristic metabolites tests, Methyl Red / Voges-Proskauer (MR/VP), iodole production, *etc.*
Various Enzymes' activity	Oxidase, catalase, urease, *etc.*
Sensitivity	Sensitivity to antibiotics, potassium cyanide, potassium sodium, antimicrobial agents, dyes, *etc.*

1.1.3.1.3 Chemotaxonomy of actinobacteria

Chemotaxonomy is based on grouping microorganisms, according to the similarities of their cellular components (Goodfellow and Minnikin, 1985). This classification rely on the chemical composition of the cell wall, such as sugars, amino acids, menaquinones, phospholipids, fatty acids, mycolic acids, muramic acid types (table 2) (Williams *et al.*, 1989). Moreover, the chemical composition of the whole cell hydrolysate can serve as fingerprinting techniques. The chemical analysis is performed after the hydrolysis of the cell by methanolysis, acid hydrolysis *etc.* (Zitouni, 2005).

The chemotaxonomy has been recommended in a polyphasic approach to apply to the species, genus, and higher taxa level (Xu *et al.*, 2007), and has been proven to be a reliable classification methods that reflects the phylogenetic relationships (Busse *et al.*, 1996).

Table 3. Chemotaxonomic markers and their cellular sites.

	Cellular site	**Composition**
Chemotaxonomy	Cell	Sugars
	Cell wall	Amino acids
	Plasmic membranes	Polar lipids
	Plasmic membranes	Menaquinones
	Plasmic membranes	Fatty acids
	Plasmic membranes	Mycolic acid

1.1.3.1.3.1 Amino acid composition

Actinobacteria at the genus level can be classified according to the morphology and cell wall composition, and it has been widely accepted since (Lechevalier and Lechevalier, 1965). The peptidoglycan of actinobacteria contains depending on the genus, different amino acids, in particular only one of the diaminopimelic acid isomers LL- or DL-(*meso*)-DAP. In this context, many chemotypes has been proposed, groups I, II, III et IV are defined by Becker *et al*. (1965), Yamaguchi (1965), and Lechevalier and Lechevalier (1970), based on the LL or

DL-DAP, and the presence or absence of glycine, groups A, B, C, D are defined by Lechevalier and Lechevalier (1970), Labeda *et al*. (1989), and Stackebrandt *et al*. (1994), based on the presence or absence of characteristic sugars (table 3) (Saker *et al.*, 2015).

Table 4. Cell wall chemotypes in *Actinomycetes* (Larpent, 2000).

Type	**Major constituent**	**Example**
Type I C	LL DAP + glycine, absence of characteristic sugars	*Streptomyces*
Type II D	Arabinose + xylose + DL DAP + glycine	*Micromonospora*
Type III B	Madurose + DL DAP	*Actinomadura*
Type III C	DL DAP et absence de sucres	*Nocardiopsis*
Type III E	Rhamnose + galactose + DL DAP	*Actinoalloteichus*
Type IV A	Arabinose + galactose + DL DAP	*Saccharomonospora*
Type V	Ornithine + lysine	*Actinomyces*
Type VI	Lysine	*Oerskovia*
Type VII	Diaminobutyric acid + glycine (lysine variably present)	*Agromyces*
Type VIII	Ornithine	*Cellulomonas*

1.1.3.1.3.2 Whole cell sugar composition

The whole cell sugar analysis is very important in the classification and identification of actinomycetes (Lechevalier, 1968). Actinomycetes can be divided into five characteristic chemotypes depending on the presence of some characteristic sugars (table 4) (Lechevalier and Lechevalier, 1970). The combination of the whole cell sugar content, and the characteristic diamino acid and some amino acids is used to describe eight wall chemotypes to discriminate between *Actinomycetes* (Lechevalier and Lechevalier, 1980).

Table 5. Sugar chemotypes in *Actinomycetes* (Lechevalier and Lechevalier, 1970; Labeda and Lechevalier, 1989).

Groupe	**Sugar content**	**Example**
Groupe A	Arabinose-galactose	*Nocardia*, *Saccharopolyspora*, *Saccharomonospora*
Groupe B	Madurose (3-*O*-methyl-d-galactose)	*Actinomadura*, *Streptosporangium*
Groupe C	Lack of characteristic sugars	*Thermomonospora*, *Thermoactinomyces*
Groupe D	Xylose-arabinose	*Actinoplane*, *Micromonospora*
Groupe E	Rhamnose-galactose	*Actinoalloteichus*

1.1.3.1.3.3 Lipid composition

Lipid profile is of major importance for the classification of *Actinomycetes*, as the amino acid and sugar composition can be insufficient for the identification and classification in many genera of *Actinomycetes*. The chemotaxonomy of lipids looks into the polar lipids

(phospholipids), the menaquinones, the fatty acids, in addition to the mycolic acid (Collins *et al*., 1977; Lechevalier and Lechevalier, 1980).

1.1.3.1.3.3.1 Phospholipids

Phospholipids are the most common polar lipids; they present an important component of the bacterial plasmic membrane, usually associated with specific proteins. The phospholipid profile is analysed by one- or two-dimensional thin-layer chromatography, and five phospholipid patterns (PI–PV) have been recognized (Table 5) (Lechevalier *et al.*, 1977).

Table 6. Phospholipid chemotypes in *Actinomycetes* (Lechevalier *et al.*, 1977).

Type	Characteristic phospholipid	Example
PI	No nitrogenous phospholipids	*Actinomadura*, *Spirullospora*
PII	Only one nitrogenous phospholipid, phosphatidyl ethanolamine	*Streptomyces*, *Pseudonokardia* *Saccharomonospora*
PIII	Phosphatidyl choline and characteristic phospholipid	*Actinopolyspora*
PIV	Glucosamine-containing phospholipids	*Amicolatopsis*
PV	Phosphatidylglycerol and glucosamine-containing phospholipid	*Nonomuraea*, *Prauserella*

1.1.3.1.3.3.2 Fatty acids

Fatty acid analysis is very important in chemotaxonomy of *Actinomycetes*, but the fatty acid composition of bacteria depends on the growth medium and culture conditions, for this reason the medium and the condition of the growth must be standardised (Smith and Norton, 1980). Fatty acids are found in the cytoplasmic membrane and lipoteichoic acids in Gram-positive bacteria (Wang and Jiang, 2016).

The distribution of fatty acid among different taxa is very characteristic, especially for very restricted fatty acids. The diagnostic of particular group depends on the length of the carbon chain, presence of saturated and unsaturated fatty acids, as well as the existence of methyl groups, cyclopropane fatty acid, and hydroxyl-fatty acid (table 6) (Kroppenstedt and Eventushenko, 2006).

Mycolic acids occur in certain high G+C Gram-positives bacteria. Mycolate structure, the length of its lateral chains, are additional taxonomic information (Tindall *et al.*, 2010).

Table 7. Fatty acids profile in actinomycetes (Kroppenstedt and Eventushenko, 2006).

Type	Branched chain fatty acids							
	Saturated	Unsaturated	Iso 14/16/18	Iso 15/17	Anteiso 15/17	10-Methyl		Cyclo propane
						17	18	
1a	+++	+++	-	-	-	-	-	-
1b	+++	+++	-	-	-	-	+	-
1c	+++	+++	-	-	-	-	-	++
2a	++	+	+++	+	(+)	-	-	-
2b	(+)	+	++	+++	+	-	-	-
2c	+	(v)	+++	+	+++	-	-	-
2d	+	+	+++	+++	+++	-	-	-
3a	+++	++	+++	(+)	(+)	(+)	+++	-
3b	+	+	+++	+++	++	++	(+)	-
3c	+	+	++	+	+	+++	(+)	-
3d	+	+	+++	++	+++	(+)	+++	-

Symbols: +: less than 1-5%; ++: between 5 and 10%; +++: between 15 and 20%; ++++: 25% or more; - : absent; (v): variable (less than 2%).

1.1.3.1.3.3.3 Menaquinones

Menaquinones are the only forms of isoprenoid quinones that exist in the cytoplasmic membrane of actinobacteria. Menaquinone patterns provide valuable information for the classification of A*ctinobacteria*, mainly in the variation in the length and the degree of hydrogenation of the C_3 isoprenyl side-chain (Collins *et al.*, 1985).

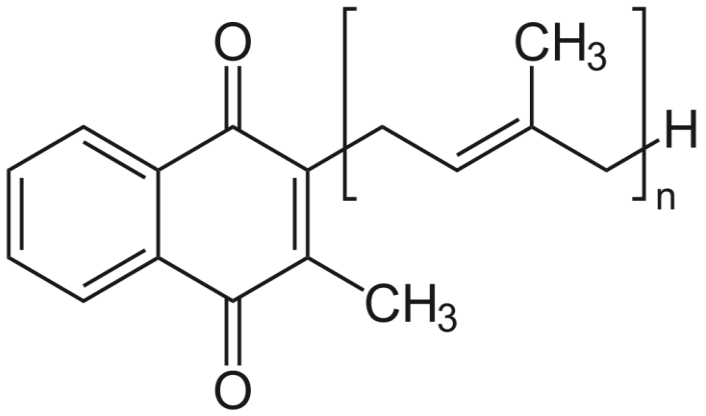


Figure 5. Menaquinones structure with n[isopren units]

Alderson *et al.* (1985), and Kroppensdedt *et al.* (1981,1985) have done an extensive work on the menaquinones analysis, and the classification of streptomycetes based on the menquinones composition. Williams *et al.* (1983) have clustered the *Actinomycetes* and related organisms accordingly (table 7).

Table 8. Menaquinones patterns of *Actinmycetes* (Williams e*t al.*, 1983).

Type	**Major menaquinone**	**Genus**
1a	MK-7	*Thermoactinomyces*
1b	MK-9	*Gordona*
2a	MK-8(H_2)	*Rhodococcus*
2b	MK-8(H_4)	*Nocardia*
2c	MK-9(H_2)	*Mycobacterium*
2d	MK-9(H_4)	*Geodermatophilus*
3a	MK-8(H_4), MK-9(H_4)	*Saccharomonospora*
3b	MK-9(H_4), MK-10(H_4)	*Actinoplanes*
4a	MK-9(H_2), MK-9(H_4), MK-9(H_6)	*Microtetraspora*
4b	MK-9(H_4), MK-9(H_6), MK-9(H_8)	*Streptomyces*
4c	MK-10(H_4), MK-10(H_6)	*Nocardiopsis*

1.1.3.2 Phylogenetic (molecular) taxonomy

Phenotypic taxonomy has many downsides and limitation. For instance, many microorganisms are poorly or unable to grow under laboratory conditions, and a phenotypic characteristic can be exhibited by many evolutionary unrelated taxa (Wilson, 1995), and closely related organisms can have divergent traits. Phenetic (non-evolutionary) taxonomy groups organisms on the basis of the phenotype, which does not give any idea about their genealogy. In contrast, phylogenetic (evolutionary) taxonomy tries to establish relationships between organisms or taxa. The concept of "evolutionary clock" came out from the realization that macromolecules can accumulate changes overtime without losing their functions (molecular chronometers), thus, the comparison of these small changes is the basis of inferring evolutionary relationships (Wilson, 1995).

Genotypic information is derived directly from the genetic material (DNA and RNA), and the advances in technology make it more reliable in term of cost, speed, and ease. The accepted molecular technics in the polyphasic approach are DNA G+C content difference, 16S rRNA gene sequence similarity, DNA–DNA hybridisation (DDH) (Rossi-Tamisier *et al.*, 2015; Chen *et al.*, 2016).

1.1.3.2.1 16S rRNA homology study

The importance of 16S rRNA rises from the fact that it is a highly conserved gene, and has been in use for taxonomy since the 1980s. It contains preserved fragments that are used to design the primers, and nine hypervariable domains (Quast *et al.*, 2013; Nguyen *et al.*, 2016).

Up to 2013, there were more than three million available 16S rRNA sequences in the public databases (Quast *et al.*, 2013), such as GenBank, and EzTaxon, and comparative tools they offer such as blast, made a massive increase in number of validated and published names, from 1800 in 1980 to almost 12500 in 2013 (Parte, 2014), and many pre-existing taxa were reclassified.

In 1994, the cut-off value at the species level was 97%, and 95% at the genus level, and it was re-evaluated at 98.65% in 2006 (Kim *et al.*, 2014). However, several authors have demonstrated that these cut-offs, initially designed to standardize the use of 16S rRNA gene sequences in taxonomy, do not apply to several genera (Rossi-Tamisier *et al.*, 2015).

1.1.3.2.2 DNA-DNA hybridisation (DDH)

DNA–DNA hybridization (DDH) is an experimental method for the determination of the overall similarity between two genomes indirectly. DDH offers a clear and objective numerical threshold for a species boundary, and has been considered "the gold standard" for bacterial species demarcation, a value of 70% DDH is accepted and used widely, and it is necessary for bacterial identification if the 16S rRNA similarity value between two strains is over 98.65% (Vandamme *et al.*, 1996; Kim *et al.*, 2014). However, due to the labour-intensive and error-prone nature of DDH experiments, there has been a continuous demand for an alternative genotype-based standards such the average nucleotide identity (ANI), and digital DDH (dDDH) which is computed using the recommended settings of the Genome-to-Genome Distance Calculator (GGDC), *etc.* (Kim *et al*., 2014; Chen *et al*., 2016).

1.1.3.2.3 Determination of the DNA G+C content

The G+C mol% is one of well accepted and used criteria in the genotypic taxonomy of prokaryotes. Its usefulness came from the fact that the external factors, growth conditions, and age of bacteria do not affect it. Additionally the G+C mol% is very similar in close organisms, and varies in distant ones (Tindall *et al.*, 2010). The G+C mol% of most actinobacteria distributes between 51 and over 70 (Stackebrandt and Ebers, 2006).

The determination methods of G+C content is determined directly or indirectly by experimental methods, such as HPLC method. However, these experimental methods can be replaced conveniently by the direct calculation from high quality, accurate whole genome sequences. The *in silico* method shows that the G+C value within a species should not be

more than 1% at most, while the value variation in the experimental method can range from 3% or even 5%, which can be attributed to experimental errors (Nouioui *et al.*, 2018).

Figure 6, summaries the different analytical approaches used in phenotypic and genotypic taxonomy, discussed above (Vandamme *et al.*, 2016).

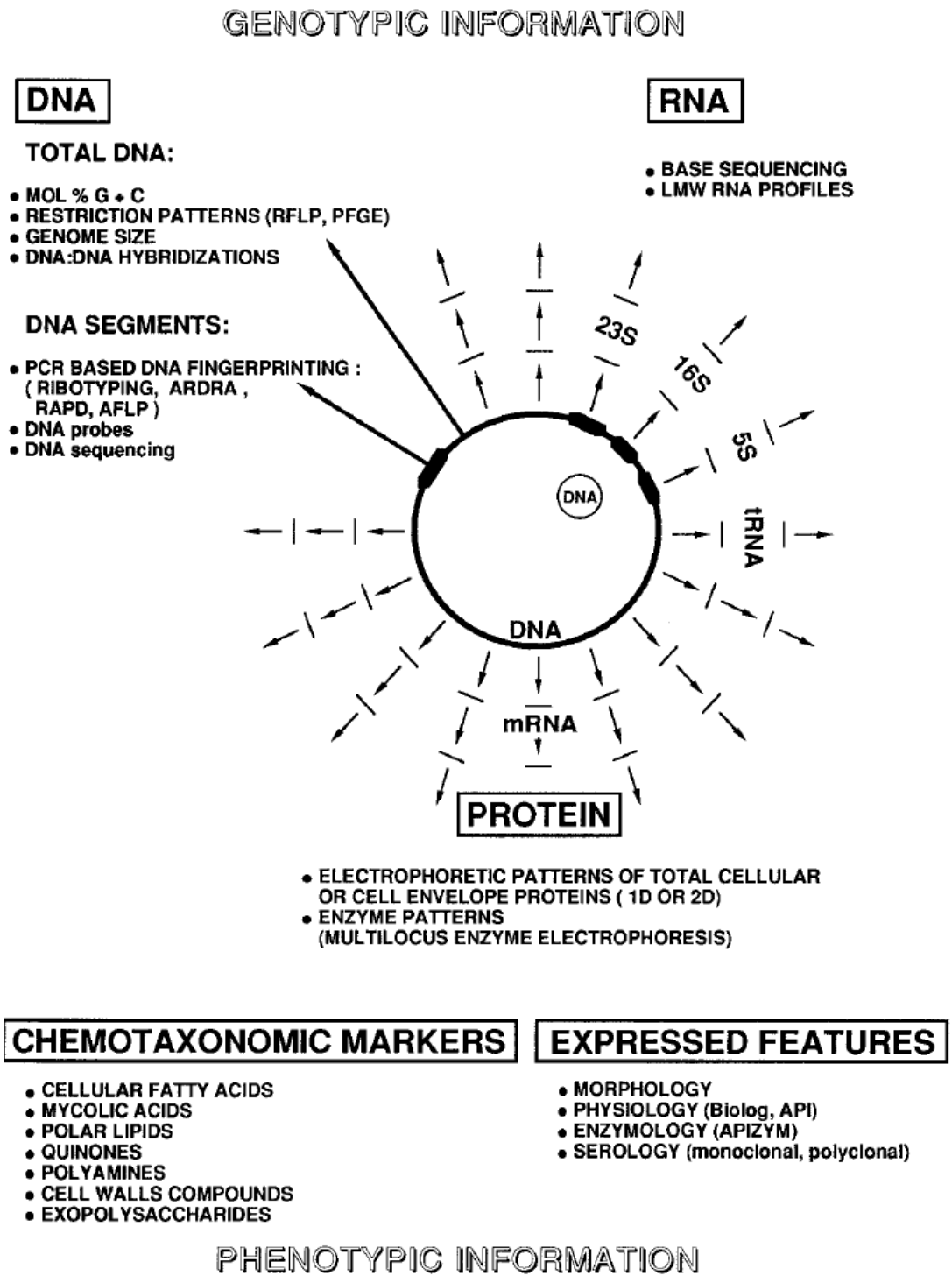


Figure 6. Schematic overview of various cellular components and different analytical approaches used in taxonomy (Vandamme *et al.*, 2016).

RFLP, restriction fragment length polymorphism; PFGE, pulsed-field gel electrophoresis; ARDRA, amplified 16S rRNA restriction analysis; RAPD, randomly amplified polymorphic DNA; AFLP, amplified fragment length polymorphism; LMW, low molecular weight; 1D, 2D, one- and two-dimensional, respectively.

1.2 Habitat and ecology of *Actinobacteria*

Actinobacteria are a group of Gram-positive, filamentous bacteria that are ubiquitous in nature (table 7), and can be found in a wide range of habitats, including soil, marine and fresh waters, in association with plants and animals (Ranjani *et al*., 2016).

In soil, *Streptomyces*, *Nocardia*, *Nocardiopsis*, and *Actinomycetes* are the most abundant soil species (Cundell and Piechoski, 2016). Actinobacteria play a key role in breaking down complex organic matter, thus, supplying the soil with recycled nutrients to plants and other microorganisms, and contributes in the geochemical cycles. Their production of humus also contributes to the formation and stability of soil aggregates, which can help improve soil structure and fertility, and most of the earthworms benefits are attributed to the their associated actinomycetes and their enzymes (Selim *et al.*, 2021). Plant growth promoting rhizobacteria (PGPR) help in nonleguminous nitrogen fixation (*Frankia*), facilitating nutrient assimilation, growth promotion, and they have protective roles by theirs antagonistic effects against insects and harmful microorganisms (Gao *et al.*, 2021; Selim *et al.*, 2021).

In aquatic environments, actinobacteria can be found as planktonic, in biofilm habitats, or mostly in the sediments (Schmidt *et al.*, 2019), where they contribute to the breakdown of dissolved organic matter and nutrient cycling, which is an important component of aquatic food webs. In addition, many actinomycetes form complex interaction with a variety of aquatic organisms, such as sponges, corals, echinoderms, and contribute to the evolution of the secondary metabolic pathway (Chen *et al.*, 2021; Jagannathan *et al.*, 2021).

Actinomycetes have also been found in association with vertebrates and invertebrates. Some actinobacteria, are commensal in the human, and animal flora. However, few opportunistic species can cause pathologies in immune-compromised individuals (Ranjani *et al.*, 2016). *Streptomyces* species also, have been isolated from the gut of termites, cockroaches, and aphids where they may help in breaking down cellulose and lignin. Moreover, *Nocardiopsis alba* was reported to play a protective role against certain drug-resistant pathogenic B*acillus* strains (Preeti *et al.*, 2010).

Actinbacteria have evolved a range of adaptations that allow them to survive in extreme and challenging habitats (figure 7). These adaptations include the production of specialized enzymes and metabolites, as well as changes in their cell membranes and other cellular components (Alshaibani, 2021). Thermophilic actinomycetes can survive and thrive at high temperatures, typically above 50°C, for example, *Thermobispora bispora* (Wang *et al.*, 1996), and *Saccharomonospora viridis* (Schuurmans *et al.*, 1956), are isolated from hot piles

of compost and manure. *Saccharomonospora halophile* is an example of halophilic actinomycetes that is isolated from Kuwaiti marsh soils (Zarban *et al.*, 2002). Acidophilic actinomycetes can tolerate low pH conditions, such as in mine drainage sites and acidic forest soils, *Streptomyces mirabilis* is one example (Brandsch *et al.*, 2022). A lot of oligotrophic actinomycetes are isolated from very nutrient-poor environments, such as deserts and arid lands, and have been the subject of numerous new bioactive metabolites prospect studies (Sayed *et al.*, 2020).

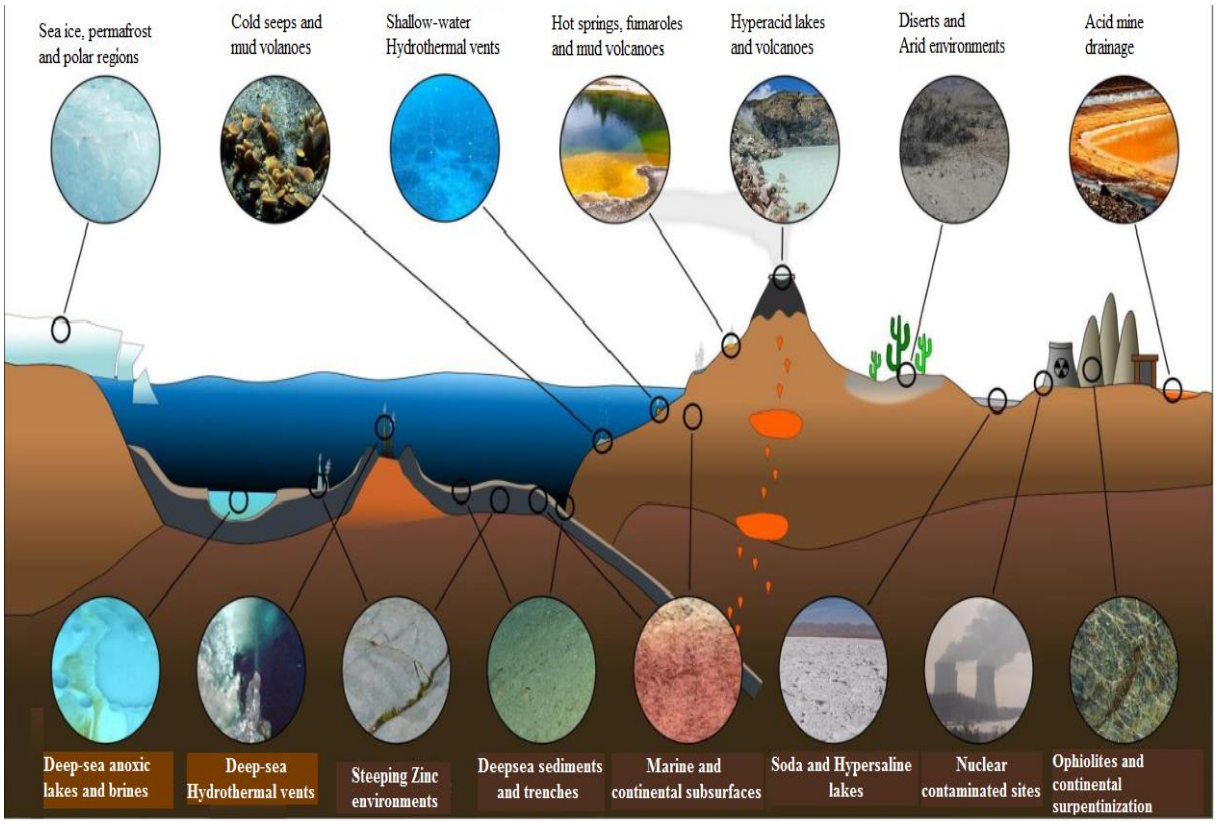


Figure 7. Cross section of Earth's crust showing the diversity of actinobacteria in extreme environments (Merino *et al.*, 2019).

The diversity and abundance of actinomycetes in different ecosystems (table 8) can be influenced by a variety of factors, including soil pH, moisture, nutrient availability, and the presence of other microorganisms. In addition, human activities such as land usange, pollution, and antibiotic use can also have significant impacts on actinomycetes communities and their ecological roles (Ranjani *et al*., 2016).

Table 9. Ecological distribution of actinobacteria (Goel *et al.*,2021).

Habitat	Area	Bacterial strain
Terrestrial	Soil	*Streptomyces* *Nocardia Streptoverticillium* *Nocardiopsis Amycolatopsis* *Micromonospora* *Actinomadura*
Aquatic	Freshwater	*Actinoplanes* *Micromonospora* *Rhodococcus Streptomyces*
	Marine	*Dietzia* *Agrococcus* *Arthrobacter* *Gordonia* *Mycobacterium* *Pseudonocardia Rhodococci.* *Streptomyces*
Extreme	Extreme environment	*Saccharomonospora* *Georgenia* *Thermotunica* *Thermobifida Amycolatopsis* *Rubrobacter*

1.3 Genus of *Saccharomonospora*

The genus *Saccharomonospora* was proposed by Nonomura and Ohara in 1971, and is a member of the family of *Pseudonocardiaceae*, and contains 15 validated species and sub species: *S. viridis* (Nonomura and Ohara, 1971) as the type species, *S. azurea* (Runmao, 1987), *S. cyanea* (Runmao *et al.*, 1988), *S. glauca* (Greiner-Mai *et al.*, 1988), *S. iraqiensis* subsp. *iraqiensis* (Ruan *et al.*, 1994; amended by Nouioui *et al.*, 2018), *S. xinjiangensis* (Jin *et al.*, 1998), *S. halophila* (Al-Zarban *et al.*, 2002), *S. iraqiensis* subsp. *paurometabolica* (Li *et al.*, 2003), *S. saliphila* (Syed *et al.*, 2008), *S. marina* (Liu *et al.*, 2010), *S. amisosensis* (Veyisoglu *et al.*, 2013), *S. oceani* (Zhang *et al.*, 2014), *S. xiaoerkulensis* (Li *et al.*, 2016) and *S. colocasiae* (Wattanasuepsin *et al.*, 2017), *S. piscinae* (tseng *et al.*, 2018).

Saccharomonospora can be distinguished from other members of the family *Pseudonocardiaceae*; by the production of single spores on aerial hyphae, the absence of sporangia-like structures, and non-fragmentation of the SM. The cell wall pattern belongs to chemotype IV (contains *meso*-DAP acid, arabinose and galactose), and the diagnostic phospholipid is phosphatidylethanolamine (phospholipid type II, with the exception of *S. xinjiangensis*). The major fatty acids are iso- and anteiso, and the main menaquinone is MK-9(H_4), while DNA G+C content varies between 68 and 74 mol% (Goodfellow *et al.*, 2012).

The aerial and vegetative mycelia are well developed and irregularly branched, but can be absent in some strains. The AM can be white, yellow-white (*S. iraqiensis* subsp. *paurometabolica* and *S. xinjiangensis*), green (*S. viridis*), or light to dark blue (*S. cyanae*); green pigmentation may occur on the SM and diffuse into the medium (Goodfellow *et al.*, 2012).

Saccharomonospora species produce mostly single spores at the tip of aerial hyphae, or paired spores on aerial hyphae (in *S. marina*, *S. saliphila* and *S. xinjiangensis*), and rarely on SM. The spores are ovoid, ellipsoidal, or round (0.7–1.1 × 1.0–1.8 μm); the surface of individual spores is smooth, warty or wrinkled (Goodfellow *et al.*, 2012) (Figure 8).

Some *Saccharomonosporae* occur in soil, and marine waters, and are halophilic, or halotolerant (Al-Zarban *et al.*, 2002; Liu *el al.*, 2010), and thermophilic *Saccharomonosporae* are found in compost piles (Schuurmans *et al.*, 1956). Many strains of *Saccharomonospora* produce degradative enzymes (Numoto *et al.*, 2018), and display antibiotic activities (Tamure and Takeda, 1975). *S. viridis* is the only member of the genus that is known to cause health issues, it is one of the causative agents of hypersensitivity pneumonitis (Raghu *et al*., 2020).

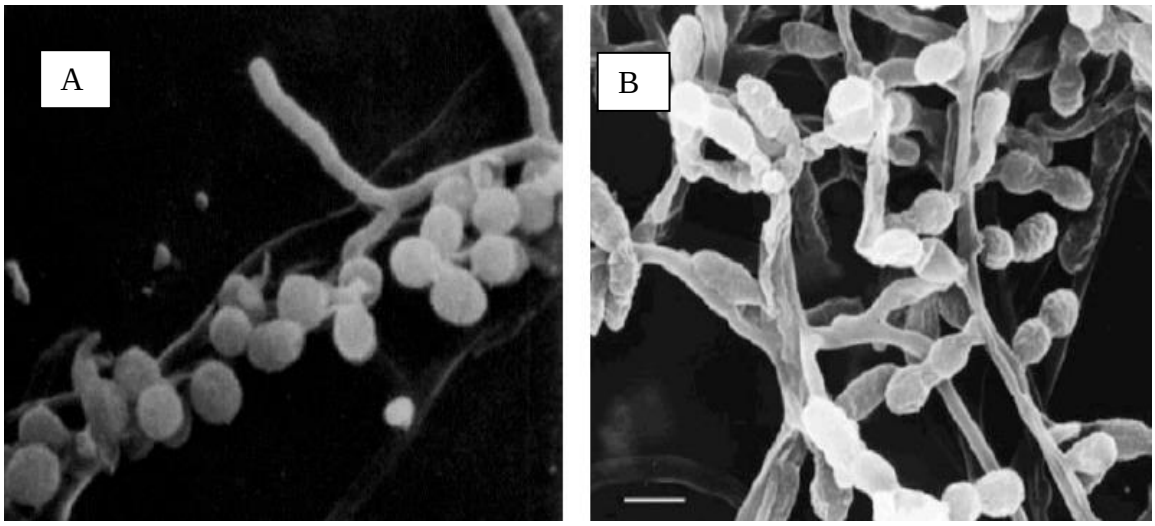


Figure 8. *Saccharomonosporae* single (A) spores and in pairs (B) (Goodfellow *et al.*, 2012).


Materials &
Methods

CHAPTER II : Materials & methods

2.1 Studied species

Fifteen validated species and subspecies of the genus *Saccharomonospora*, have been selected for this study. A brief description of the species is listed in the alphabetical order:

- ***Saccharomonospora amisosensis*:** The type strain DS3030^T (= DSM 45685^T = KCTC 29069^T = NRRLB-24885^T), was isolated from deep sediment from the southern Black Sea coast, Turkey. Aerobic, Gram-positive actinomycete, non-motile, form branched SM that produces single spores, or pairs and short chains of spores. The DNA G+C content of the type strain is 68.9 mol% (Veyisoglu *et al.*, 2013).

- ***Saccharomonospora azurea*:** The Type strain NA-128^T (= SIA 86128^T), was isolated from a soil sample collected at Guangyuan City, Sichuan, China. Aerobic, Gram-positive actinomycete, mesophilic, nonfragmenting SM, no sporangium, single oval or round spores with a smooth surface, mainly on AM, very short or sessile sporophores. The colour of the AM is azure on oatmeal agar and Czapek sucrose agar, with no distinct soluble pigment (Runmao *et al.*, 1987). The DNA G+C content of the type strain is 70.08 mol% (Klenk *et al.*, 2012).

- ***Saccharomonospora colocasiae*:** The type strain S265^T (= TBRC 7235^T = NBRC 112945^T), was isolated from the rhizosphere of *Colocasia esculenta* that had been collected from Bangmod district, Jomthong, Bangkok, Thailand. Aerobic, Gram-positive, mesophilic actinomycete with branched SM and AM, with single wrinkled and spherical spores. Abundant green AM on ISP 2, ISP 4 and nutrient agar, with no diffusible pigments. The DNA G+C content of the type strain is 69 mol% (Wattanasuepsin *et al.*, 2017).

- ***Saccharomonospora cyanea:*** The type strain NA-134^T (= SIIA 86134^T = ATCC 43724^T), was isolated from soil samples collected at Guangyuan, Sichuan, China. Aerobic, gram-positive actinomycete, mesophilic, nonfragmenting SM, with no sporangium, single oval to ellipsoidal warty spores mainly on AM. Very short or sessile sporophores. The colour of the AM is light to dark blue on oatmeal agar and Czapek sucrose agar; with no distinct soluble pigment (Runmao *et al.*, 1988). The DNA G+C content of the type strain is 69.74 mol% (Meier-Kolthoff *et al.*, 2013).

- ***Saccharomonospora glauca*:** The type strain K62 ^{T} (= DSM 43769^{T}), was isolated from compost pile in Germany. Branching, nonfragmenting AM and SM. single smooth round to ovate spores on aerial hyphae. Light green to bluish green (turquoise) AM, dark green SM, diffusible pigments on GC agar and GYM (Glucose, Yeast extract, Malt extract) agar (Greiner-Mai *et al*., 1988). The G+C content of the type-strain genome is 69.1 mol% (Nouioui *et al.*, 2018).

- ***Saccharomonospora halophila*:** The type Strain 8^{T} (= DSM 44411^{T} = NRRL B-24125^{T}) was isolated from salt marsh soil in Kuwait. Aerobic, Gram-positive, non-motile halophilic actinomycete, that forms light blue AM (Al-Zarban *et al.*, 2002). The G+C content of the type-strain genome is 70.9 mol% (Nouioui *et al.*, 2018).

- ***Saccharomonospora iraqiensis* subsp. *iraqiensis*:** The type strain is IQ-H1^{T} (= DSM 44640^{T} = JCM 9891^{T} = NBRC 103187^{T}), was isolated from saline soil samples in Iraq. Mesophilic and moderately halophilic. Small, thin, elevated or convex colonies, yellow to brownish in colour, with no diffusible pigment. The spore mass is white and abundant on solid medium (10 or 15% NaCl [wt/vol]). SM is well developed and branched, rarely fragmented. Short chains of smooth and spherical spores in AM (1 to 15 conidia) (Ruan *et al.*, 1994). The DNA G+C content of the type-strain genome is 71.5 mol% (Nouioui *et al.*, 2018).

- ***Saccharomonospora iraqiensis* subsp. *paurometabolica*:** the type strain is YIM 90007^{T} (= CCTCC AA001018^{T} = CCRC 16315^{T} = DSM 44619^{T}), was isolated from saline soil from the Xinjiang province, in the western China. Well-developed white AM on most media, green-yellow on nutrient agar, and poorly developed on inorganic salt/starch agar and potato agar. Sporulation is good on ISP2, ISP5, moderate on ISP3 and poor on ISP4. SM is well developed on most test media, with fluctuating colours according to media. Single non-motile smooth (or wrinkled) spores on AM, and sometimes single spores on SM. the DNA G+C content is 71 mol% (Li *et al.*, 2003).

- ***Saccharomonospora marina*:** The type XMU15^{T} (= KCTC 19701^{T} = CCTCC AA 209048^{T}) was isolated from an ocean sediment sample collected from Zhaoan Bay in the East China sea, Fujian province, China. Aerobic, Gram-positive actinomycete. Non-motile smooth or wrinkled single (pairs, or in short chain) spores are produced on the branched AM. The DNA G+C content of the type strain is 68.1 mol% (Liu *et al.*, 2010).

- ***Saccharomonospora oceani*:** The type strain YIMM11168^T (= DSM 45700^T = JCM 18128^T) was isolated from marine sample collected in Little Andaman, India. Gram-positive, aerobic actinomycete. Good growth on many media with branched non fragmented pale yellow to orange-yellow SM and white AM, but no growth on ISP 4 and ISP 5 agar. Ovate wrinkled single or paired spores form on AM and SM, and occasionally single spores are formed on long sporophores. The G+C content of the genomic DNA is 71.4 mol% (Zhang *et al.*, 2014).

- ***Saccharomonospora piscinae* :** The type strain 06168H-1^T (= BCRC 16893^T = KCTC 19743^T), was isolated from dried fishpond sediment of Kouhu area, in southern Taiwan. Gram positive, aerobic and mesophilic actinomycete. Form branched, non-fragmented olive green to greyish green SM. Short chains of 3 to 10 non-motile smooth ovate spores are formed on pale green to greenish grey AM. Sporulation only occurs on oatmeal agar, and ISP4, nutrient agar and Czapek's sucrose agar. The DNA G+C content of the strain is 70.6 mol% (Tsang *et al.*, 2018).

- ***Saccharomonospora saliphila*:** The type strain YIM 90502^T (= KCTC 19234^T = DSM 45087^T), was isolated from soil collected from Gulbarga, Karnataka Province, India. Well-developed greyish to reddish-grey AM on most media with good sporulation, but no growth on oatmeal agar or nutrient agar. Non-motile single or paired non motile smooth or wrinkled spores are formed on AM. The DNA G+C content of the strain is 71.8 mol% (Syed *et al*., 2008).

- ***Saccharomonospora viridis* :** The type strain is P101^T (= ATCC 15386^T = CCUG 5913^T = DSM 43017^T = NBRC 12207^T = JCM 3036^T = NRRL B-3044^T = VKM Ac-681^T), and is the type species of the genus *Saccharomonospora*. It is frequently found in hot compost and hay. Its readily dispersed spores can cause farmer's lung disease, and humidifier fever. It is interestingly Gram-stain negative, aerobic, catalase- and oxidase-positive actinomycetes. Produces branched, sometimes curved endings green AM, and yellow SM on ISP2 (Shin *et al.*, 2017). The DNA G+C content of the type-strain is 67.3% (Nouinoui *et al.*, 2018).

- ***Saccharomonospora xiaoerkulensis* :** The type strain TRM 41495^T (= CCTCC AA 2015038^T = KCTC 39727^T), was isolated from a silt sample from Xiaoerkule lake in Xinjiang province. Aerobic, Gram-positive actinomycete, abundant AM with smooth surface and

irregular branches, with ovate and smooth surface spores. The G+C content of the genomic DNA is 72.9 mol% (Li *et al.*, 2016).

- ***Saccharomonospora xinjiangensis* :** The type strain XJ-54^T (= CCTCC AA97021^T) was Isolated from soil in Xinjiang, China. Light yellowish vegetative hyphae. The AM is yellow-white on most media, and light green-grey on Czapek agar. Longitudinal pairs of spores are formed on both SM and AM, and occasionally single spores are born on AM. Observed diffusible light yellow-brown pigments on potato extract-glucose agar and non on tyrosine agar (Jin *et al.*, 1998). The G+C content of the genomic DNA is 68.9% (Nouinoui *et al.*, 2018).

2.2 Chemotaxonomic analysis

In this study, we relied on a chemotaxonomic analysis based on cellular components, and more precisely on the profile of sugars, amino acids, polar lipids, fatty acids and menaquinones.

The data about these components is gathered from systematic review of published scientific literature about the studies species and relative taxa. Each component is given a binary value (1/0) in regard to its presence or absence, respectively. The final data are presented in a matrix format where the rows refer to the studied species, and the columns represent the cellular components in question.

2.2.1 Similarity calculation

As many characteristics as possible are considered when measuring distance, similarity, or dissimilarity between two sets, and various statistical methods can be used. In this study, two statistical coefficients are used to calculate the similarity between the species using *S. piscinae* as a reference. The coefficients used are the coefficient of *Jaccard*, and the coefficient of *Kulczynski-2*, and both have the attribute of evading the double-zeros (0,0) in the data set (Legendre and Legendre, 1998).

2.2.1.1 Coefficient of *Jaccard*

The *Jaccard* similarity coefficient or index, also known as the coefficient of community, was developed by the Swiss botanist Paul Jaccard (1868–1944). The coefficient measures the similarity between two sets, and is defined as the size of the intersection of the

two sets divided by the size of the union of the two sets (Cheetham and Hazel, 1969; Legendre and Legendre, 1998).

The *Jaccard* distance, d_J, is given as $\boldsymbol{J = \frac{a}{a+b+c}}$, where a, b, c, and d are shown in the following matrix of distances:

Matrix of distance

	1	**0**
1	**a**	**b**
0	**c**	**d**

a = (1, 1); **b** = (0, 1); **c** = (1, 0); **d** = (0, 0).

In this example, we calculated the similarity between species E1 and E2 from the following table:

Exemplary table of matrix:

Variables / **Species**	C1	C2	C3	C4	C5
E1	1	1	0	0	0
E2	0	1	1	1	0
E3	0	0	1	0	1
E4	1	1	1	1	1

The similarity between E1 and E2: a (1, 1) = 1; b (0, 1) = 2; c (1, 0) = 1; d (0, 0) = 1.

$J = \frac{1}{1+2+1}$ = **0,25** → The similarity between E1 and E2 is **25%**.

2.2.1.2 Coefficient of *Kulczynski-2*

Kulczynski-2 is another coefficient for binary data, developed by the Polish botanist Stanisław Kulczyński (1895-1975). It is commonly used in taxonomy and bioassociation studies, which is the arithmetic mean probability that if one object has an attribute, the other object has it too (Cheetham and Hazel, 1969; Legendre and Legendre, 1998).

$$\mathbf{Kul} = \left(\frac{a}{a+b} + \frac{a}{a+c}\right)/2$$

The *Kulczynski-2* distance is calculated as shown in the following example:

Matrix of distance

	1	**0**
1	**a**	**b**
0	**c**	**d**

a = (1, 1); **b** = (0, 1); **c** = (1, 0); **d** = (0, 0).

Exemplary table of matrix:

Variables / **Species**	C1	C2	C3	C4	C5
E1	1	1	0	0	0
E2	0	1	1	1	0
E3	0	0	1	0	1
E4	1	1	1	1	1

The similarity between E1 and E2 : a (1, 1) = 1; b (0, 1) = 2; c (1, 0) = 1; d (0, 0) = 1.

Kul = [(1/1+2) + (1/1+1)]/2 = 0.416 → The similarity between E1 and E2 is **41.6%**

The similarity between E1 and E2 according to *Jaccard* index is **25%**.

The similarity between E1 and E2 according to *Kulczynski-2* index is **41.6%**.

2.2.1.3 PAleontological STatistics 4.13 (*PAST 4.13*)

PAleontological STatistics 4.13 (*PAST 4.13*) is paleontological statistics analysis software widely used for data analysis and visualization in various fields of biology, including palaeontology, ecology, and evolutionary biology. *PAST 4.13* supports a wide variety of data types, including numerical, categorical, and binary (presence-absence) data. It provides numerous functions for data manipulation, summary statistics, hypothesis testing, ordination techniques, clustering, and more (Hammer *et al.*, 2001).

Chemotaxonomic data are properly formatted with species names as rows and the chemotaxonomic features as columns. The similarity matrices are calculated based on *Jaccard* Index, and *Kulczynski* Index, and the similarity order of the species to *S. piscinae* is calculated. The last step is the clustering and the generation of dendrograms, by the Neighbour-Joining method.

Figure 9: *PAST 4.13*

2.3 Molecular analysis

The molecular study of the species is based on the 16S rRNA gene sequences, the phylogenetic distances are calculated in comparison to *S. piscinae* (Tseng *et al.*, 2018), for being the last validated and published species of the genus *Saccharomonospora*. A phylogenetic tree is generated using *MEGA 11* by Neighbour-Joining method, and the evolutionary distances are compared with the similarity order obtained from EZbiocloud.

Neighbour Joining (NJ) clustering (Saitou and Nei, 1987) is a method that was originally developed for phylogenetic analysis as an alternative for hierarchical cluster analysis. NJ has the advantage over Unweighted Pair-Group Method with Arithmetic Averaging (UPGMA) that it does not assume equal rates of evolution, so that branch lengths are proportional to amount of change (Hammer *et al.*, 2001).

2.3.1 Molecular Evolutionary Genetics Analysis (*MEGA 11*)

Molecular Evolutionary Genetics Analysis version 11 (*MEGA11*) (Tamura *et al.*, 2021), is a widely used and powerful software tool for conducting molecular evolutionary analysis, in the field of molecular biology and bioinformatics. MEGA supports a wide range of sequence formats (FASTA, GenBank), and provides advanced sequence alignment algorithms, including Muscle and ClustalW, to ensure accurate alignment of sequences for subsequent analysis. In addition, MEGA has the ability to construct phylogenetic trees and offers multiple tree construction methods (Neighbour Joining, maximum likelihood), allowing researchers to explore the evolutionary relationships between species or groups of organisms. MEGA also provides tools for evaluating the reliability of phylogenetic trees, such as bootstrap analysis and branch support estimation. The *MEGA* version used in this work is 11.0.13 (Tamura *et al.*, 2021).

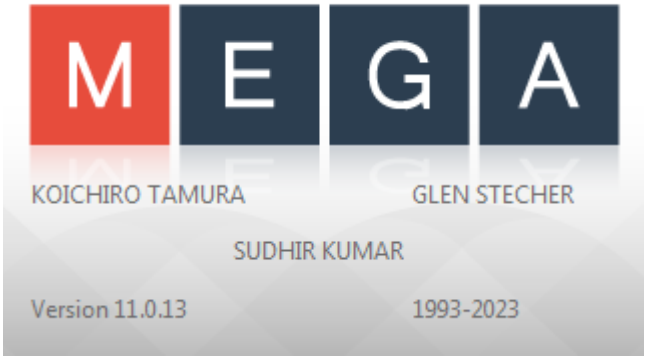


Figure 10. *MEGA 11* Tool

2.3.2 Sequences alignments and phylogenetic tree construction

The 16S rRNA gene sequences of the studied species and subspecies of the genus *Saccharomonospora*, and an outgroup species *Actinopolyspora algeriensis* (Meklat *et al.*, 2013), are retrieved from LPSN (List of Prokaryotic names with Standing in Nomenclature) FASTA format.

Figure 11. LPSN data base.

The Sequences are uploaded to MEGA 11 software, than pairwise and multiple alignments were performed using ClustalW, to ensure that they are in the correct reading frame and properly aligned. The gap opening penalty and extended gap penalty are set to 15, and 6.66, respectively, for both pairwise and multiple alignments.

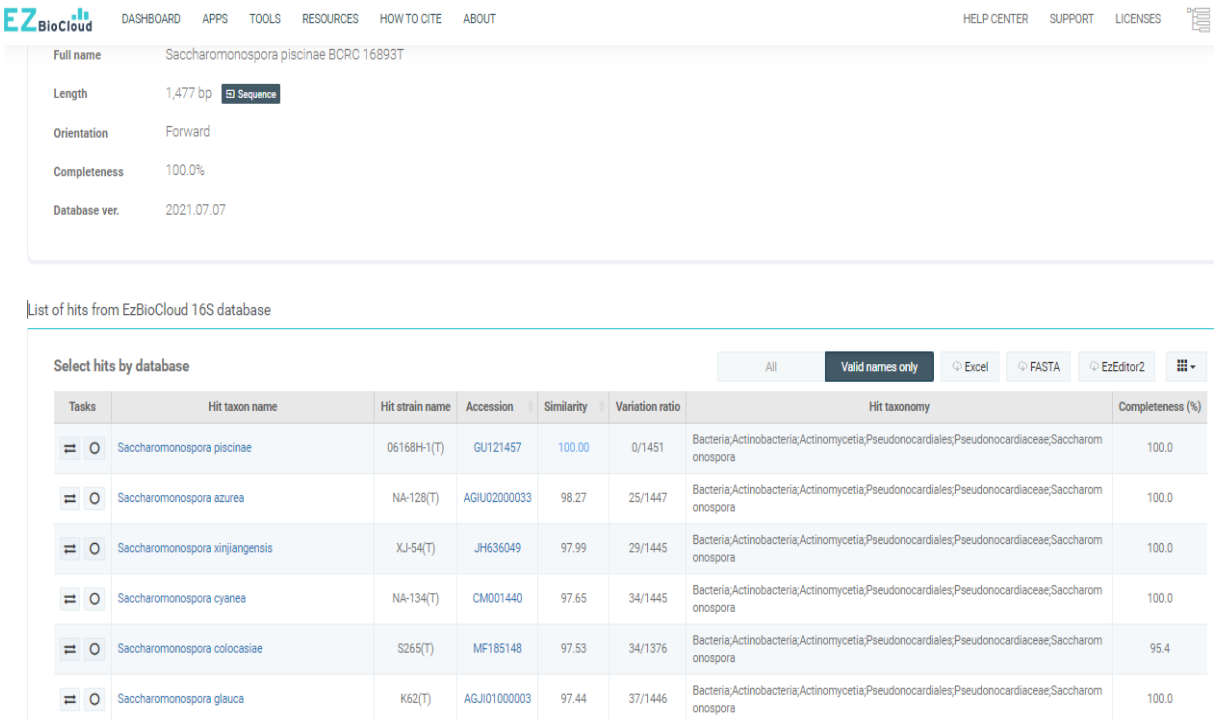
The phylogenetic tree is constructed using the Neighbour-Joining method (Saitou and Nai, 1987).

Figure 12. 16S rRNA gene sequences in *MEGA 11* after alignment.

2.3.3 Molecular similarity based on EZbiocloud

EZbiocloud is an online platform that offers a variety of bioinformatics tools and resources for microbiology research and analysis. It provides a user-friendly interface and a range of powerful features to support researchers in analysing data, particularly focusing on 16S rRNA gene sequencing and taxonomic classification

For the assessment of the similarity order of the studied species to *S. piscinae*, based on their 16S rRNA gene, the 16S rRNA gene sequence *S. piscinae* was retrieved from LPSN, and uploaded to the "16S-based ID" web application of the EZBioCloud platform. The platform assign the sequence to *S. piscinae*, and provide a list of hits of similarity order given in percentage. This similarity order to *S. piscinae*, will be compared with the similarity order given by the molecular approache given by N J in *MEGA11*.



EZ BioCloud DASHBOARD APPS TOOLS RESOURCES HOW TO CITE ABOUT HELP CENTER SUPPORT LICENSES

Full name	Saccharomonospora piscinae BCRC 16893T
Length	1,477 bp Sequence
Orientation	Forward
Completeness	100.0%
Database ver.	2021.07.07

List of hits from EzBioCloud 16S database

Select hits by database — All | Valid names only | Excel | FASTA | EzEditor2

Tasks	Hit taxon name	Hit strain name	Accession	Similarity	Variation ratio	Hit taxonomy	Completeness (%)
	Saccharomonospora piscinae	06168H-1(T)	GU121457	100.00	0/1451	Bacteria;Actinobacteria;Actinomycetia;Pseudonocardiales;Pseudonocardiaceae;Saccharomonospora	100.0
	Saccharomonospora azurea	NA-128(T)	AGIU02000033	98.27	25/1447	Bacteria;Actinobacteria;Actinomycetia;Pseudonocardiales;Pseudonocardiaceae;Saccharomonospora	100.0
	Saccharomonospora xinjiangensis	XJ-54(T)	JH636049	97.99	29/1445	Bacteria;Actinobacteria;Actinomycetia;Pseudonocardiales;Pseudonocardiaceae;Saccharomonospora	100.0
	Saccharomonospora cyanea	NA-134(T)	CM001440	97.65	34/1445	Bacteria;Actinobacteria;Actinomycetia;Pseudonocardiales;Pseudonocardiaceae;Saccharomonospora	100.0
	Saccharomonospora colocasiae	S265(T)	MF185148	97.53	34/1376	Bacteria;Actinobacteria;Actinomycetia;Pseudonocardiales;Pseudonocardiaceae;Saccharomonospora	95.4
	Saccharomonospora glauca	K62(T)	AGJI01000003	97.44	37/1446	Bacteria;Actinobacteria;Actinomycetia;Pseudonocardiales;Pseudonocardiaceae;Saccharomonospora	100.0

Figure 13. Identification results of *S. piscinae* and similarity table in EZbiocloud.

Results & discussion

CHAPTER III : Results & discussion

3.1 Chemotaxonomy

The chemotaxonomy results of the cellular components, are presented down below in separate tables according to sugars, amino acids, menaquinones, polar lipids, and fatty acids, with reference to the relevant studies used in this work. Such characteristic components are only recorded as present or absent (+ or -), as no quantitative references are taken in consideration.

3.1.1 Sugars content analysis

The results (table 9) show clearly that galactose and arabinose can be considered as biochemical marker sugars, as they are present in all the species of the genus *Saccharomonospora*. This finding confirms the position of the *Saccharomonospora* within type A whole-cell sugars chemotype in the studies of Lechevalier & Lechevalier (1961). Ribose, glucose, and mannose are present variably with 53.33%, 33.33%, and 26.66% respectively. The species *S. amisosensis* is characterised by the presence of xylose, while *S. piscinae* by the presence of madurose.

The presence of other sugars, as presented in table 9, is more or less characteristic.

Table 9. Whole-cell sugar content of *Saccharomonosporae*.

Species	Glu	Man	Gal	Rib	Ara	Xyl	Mad	Unk. S.
S. amisosensis (Veyisoglu *et al.*, 2013)	+	-	+	-	+	+	-	-
S. azurea (Runmao, 1987)(Klenk *et al.*, 2012)(Wattanasuepsin *et al.*, 2017)	-	-	+	-	+	-	-	-
S. colocasiae (Wattanasuepsin *et al.*, 2017)	+	-	+	+	+	-	-	-
S. cyanea (Runmao *et al.*, 1988)(Meier-Kolthoff *et al.*, 2013)	-	-	+	-	+	-	-	-

Table 9. – Continued- Whole-cell sugar content of *Saccharomonosporae*.

S. glauca (Greiner-Mai *et al.*, 1988)	-	-	+	-	+	-	-	-
S. halophila (Al-Zarban *et al.*, 2002)(Tang *et al.*, 2011)	+	+	+	+	+	-	-	+
S. iraqiensis subsp. *iraqiensis* (Ruan *et al.*, 1994)	-	-	+	+	+	-	-	-
S. iraqiensis subsp. *paurometabolica* (Li *et al.*, 2003)	-	-	+	+	+	-	-	-
S. marina (Liu *et al.*, 2010)(Veyisoglu *et al.*, 2013)	-	-	+	+	+	-	-	-
S. oceani (Zhang *et al.*, 2013)	+	-	+	-	+	-	-	-
S. piscinae (Tseng *et al.*, 2018)	+	+	+	+	+	-	+	-
S. saliphila (Syed *et al.*, 2008)	-	-	+	-	+	-	-	-
S. viridis (Nonomura and Ohara, 1971)	-	+	+	+	+	-	-	-
S. xiaoerkulensis (Li *et al.*, 2016)	-	+	+	+	+	-	-	-
S. xinjiangensis (Jin *et al.*, 1998)	-	-	+	-	+	-	-	-
Frequency	5/15	4/15	15/15	8/15	15/15	1/15	1/15	1/15
	33.33%	**26.66%**	**100%**	**53.33%**	**100%**	**6.66%**	**6.66%**	**6.66%**

Glu, Glucose; **Man**, Mannose; **Gal**, Galactose; **Rib**, Ribose; **Ara**, Arabinose; **Xyl**, Xylose; **Mad**, Madurose; **Unk. S.**, Unkown sugar.

3.1.2 Amino acids content analysis

Amino acids and peptidoglycan analysis revealed the constant presence of the *meso* form of the diaminopimelic acid in all the strains. Therefore, the presence of *meso*-DAP can be undoubtedly considered as a chemical marker for the studied strains. In fact, this confirms the description of the genus *Saccharomonospora* as having both type A sugar pattern (galactose and ribose), and the *meso*-DAP amino acid in the peptidoglycan, makes this genus fits into type IV cell wall chemotype as proposed by Becker et colleague (1965), which led Nonomura and Ohara (1971)

subsequently to propose the creation of the genus *Saccharomonospora*. The other amino acids are more or less present in the analysed strains and could not be used for differentiation purposes (Table 10). However, *S. iraqiensis* subsp. *iraqiensis*, is the only member of this genus that contains a trace amount of LL-Dap acid in the peptidoglycan, which could be considered as a distinctive feature (Ruan *et al.*, 1994). Also *S. colocasiae* can be characterised as having N-acetylmuramic acid in the peptidoglycan. (Wattanasuepsin *et al.*, 2017).

S. viridis is unusually Gram-stain negative, but it has a typical mycelium morphology of Gram-positive actinomycetes (Embley *et al.*, 1988; Pati *et al.*, 2009; Wattanasuepsin *et al.*, 2017), the Gram stain coloration was re-evaluated, and confirmed by the KOH method (Shin *et al.*, 2017). The absence of teichoic acid confirms the Gram-stain negative reaction in *S. viridis* (Pati *et al.*, 2009).

S. colocasiae is the only species that contains N-acetylmuramic acid, and *S. viridis* is the only species that contains the amino acid glycine, which could characteristic features of the two species.

Becker and colleagues (1964), stated that the Type IV cell wall strains, contain in addition to *meso*-DAP, arabinose and galactose, all contain glucosamine, muramic acid, alanine, glutamic acid, as major components, only if sited otherwise, but the results of more recent studies presented in table (10) show a great variability in the presence of such molecules.

Table 10. Amino acid and peptidoglycan content analysis of *Saccharomonosporae*.

Species	Glu	Ala	Gly	GlcN	LL-DAP	*meso*-DAP	Mur	Mur NAc
S. amisosensis (Veyisoglu *et al.*, 2013)	+	+	-	+	-	+	+	-
S. azurea (Runmao, 1987)(Klenk *et al.*, 2012)(Wattanasuepsin *et al.*, 2017)	+	+	-	+	-	+	+	-
S. colocasiae (Wattanasuepsin *et al.*, 2017)	-	-	-	-	-	+	-	+
S. cyanea (Runmao *et al.*, 1988)(Meier-Kolthoff *et al.*, 2013)	+	+	-	+	-	+	-	-

Table 10. –Continued- Amino acid and peptidoglycan content analysis of *Saccharomonosporae*.

S. glauca (Greiner-Mai *et al.*, 1988)	-	-	-	-	-	+	-	-
S. halophila (Al-Zarban *et al.*, 2002)(Tang *et al.*, 2011)	-	-	-	+	-	+	-	-
S. iraqiensis subsp. *iraqiensis* (Ruan *et al.*, 1994)	-	-	-	-	+	+	-	-
S. iraqiensis subsp. *paurometabolica* (Li *et al.*, 2003)	-	-	-	-	-	+	-	-
S. marina (Lieu *et al.*, 2010)(Veyisoglu *et al.*, 2013)(Zhang *et al.*, 2013)	-	-	-	-	-	+	-	-
S. oceani (Zhang *et al.*, 2013)	-	-	-	-	-	+	-	-
S. piscinae (Tseng *et al.*, 2018)	-	-	-	-	-	+	-	-
S. saliphila (Syed *et al.*, 2008)	-	-	-	-	-	+	-	-
S. viridis (Embley *et al.*, 1988)(*Pati*, 2009)(Wattanasuepsin *et al.*, 2017)	+	+	+	-	-	+	-	-
S. xiaoerkulensis (Li *et al.*, 2016)	-	-	-	-	-	+	-	-
S. xinjiangensis (Jin *et al.*, 1998)(Lieu *et al.*, 2010)	+	+	-	+	-	+	+	-
Frequency	**5/15**	**5/15**	**1/15**	**5/15**	**1/15**	**15/15**	**3/15**	**1/15**
	33.33%	**33.33%**	**6.66%**	**33.33%**	**6.66%**	**100%**	**20%**	**6.66%**

Glu: Glutamate; **Ala**: Alanine; **Gly**: Glycine; **GlcN**: Glucosamine; **LL-DAP**: LL-Diaminopimelic acid; ***meso*-DAP**: *meso*-Diaminopimelic acid; **Mur**: Muramic acid; **Mur NAc**: N-acetylmuramic acid.

3.1.3 Menaquinones content analysis

The menaquinones analysis profile showed clearly that the most representative menaquinones in the species of the *Saccharomonospora* are MK-8(H_4) and MK-

9(H_4), this finding is in accordance with the work of Kroppenstedt (1985) who extended the chemotaxonomic description of this genus, with the exception of *S. iraqiensis* subsp. *paurometabolica* that lacks MK-8(H_4), and contains MK-9(H_2) instead. *S. xinjiangensis* (Jin *et al.*, 1998) can be distinguished as having MK-7(H_2), and also *S. iraqiensis* subsp. *iraqiensis* by having MK-10(H_4) (Ruan *et al.*, 1994). Whereas MK-9(H_2), MK-8(H_6) and MK-7(H_4) are variably present as minor components (Table 11).

Table 11. Menaquinones content analysis of *Saccharomonosporae*.

Species	MK-7(H2)	MK-7(H4)	MK-8(H_2)	MK-8(H_4)	MK-8(H6)	MK-9(H_2)	MK-9(H_4)	MK-9(H_6)	MK-10(H_4)
S. amisosensis (Veyisoglu *et al.*, 2013)	-	+	-	+	-	-	+	-	-
*S. azurea (*Klenk *et al.*, 2012)	-	-	-	+	-	-	+	-	-
S. colocasiae (Wattanasuepsin *et al.*, 2017)	-	-	-	+	-	-	+	-	-
S. cyanea (Runmao *et al.*, 1988)	-	-	-	+	-	-	+	-	-
S. glauca (Greiner-Mai *et al.*, 1988)	-	-	-	+	-	-	+	-	-
S. halophila (Al-Zarban *et al.*, 2002)	-	-	-	+	-	-	+	+	-
S. iraqiensis subsp. *iraqiensis* (Ruan *et al.*, 1994)	-	-	+	+	-	+	+	-	+
S. iraqiensis subsp. *paurometabolica* (Li *et al.*, 2003)	-	-	-	-	-	+	+	-	-
S. marina (Veyisoglu *et al.*, 2013)	-	-	-	+	-	-	+	-	-
S. oceani (Zhang *et al.*, 2013)	-	-	-	+	-	-	+	-	-
S. piscinae (Tseng *et al.*, 2018)	-	-	-	+	-	-	+	+	-
S. saliphila (Syed *et al.*, 2008)	-	-	-	+	-	-	+	-	-

Table 11. –Continued-Menaquinones content analysis of *Saccharomonosporae*.

S. viridis (Embley et al., 1988)(Wattanasuepsin *et al.*, 2017)	-	-	-	+	+	-	+	+	-
S. xiaoerkulensis (Li *et al.*, 2016)	-	-	-	+	-	+	+	-	-
S. xinjiangensis (Jin *et al.*, 1998)	+	+	-	+	-	+	+	-	-
Frequency	**1/15**	**2/15**	**1/15**	**14/15**	**3/15**	**4/15**	**15/15**	**3/15**	**1/15**
	6.66 %	**13.33 %**	**6.66%**	**93.33 %**	**20%**	**26.66 %**	**100%**	**20 %**	**6.66%**

3.1.4 Polar lipids analysis

The results in table 12, showed that phosphatidylethanolamine (PE) is the most represented phospholipid (12/15), followed by diphosphatidylglycerol (DPG) (11/15), phosphatidylinositol (PI) 10/15, and phosphatidylglycerol (PG) 9/15. This finding is well corresponding to the type II phospholipid pattern (Lechevalier *et al.*, 1981; Embly *et al.*, 1988). However; it is important to mention that we have considered that phospholipids of *S. cyanae*, and *S. azurea* as absent, for the reason that there is no available data on the phospholipid content for *S. cyanae*, and Klenk *et al.* (2012) has reported the same remark for *S. azurea*.

Table 12. Phospholipid content analysis of *Saccharomonosporae*.

Species	PE	DPG	PI	PG	HPE	PC	PME	LPE
S. amisosensis (Veyisoglu *et al.*, 2013)	+	+	+	-	-	-	-	-
S. azurea (Klenk *et al.*, 2012)	-	-	-	-	-	-	-	-
S. colocasiae (Wattanasuepsin *et al.*, 2017)	+	+	-	-	-	-	-	-
S. cyanea (Runmao *et al.*, 1988)	-	-	-	-	-	-	-	-
S. glauca (Greiner-Mai *et al.*, 1988)	+	-	-	-	+	-	-	+
S. halophila (Al-Zarban *et al.*, 2002)	+	+	+	-	+	-	+	+

Table 11. Phospholipid content analysis of *Saccharomonosporae.*

S. iraqiensis subsp. *iraqiensis* (Ruan *et al.*, 1994)	-	+	-	+	-	-	-	-
S. iraqiensis subsp. *paurometabolica* (Li *et al.*, 2003)	+	+	+	+	+	-	-	-
S. marina (Lieu *et al.*, 2010)	+	+	+	+	-	-	-	-
S. oceani (Zhang *et al.*, 2013)	+	+	+	+	-	-	+	-
S. piscinae (Tseng *et al.*, 2018)	+	+	-	+	+	+	+	-
S. saliphila (Syed *et al.*, 2008)	+	+	+	+	-	-	-	-
S. viridis (Embley *et al.*, 1988)	+	+	+	+	+	-	-	+
S. xiaoerkulensis (Li *et al.*, 2016)	+	+	+	+	-	+	-	-
S. xinjiangensis (Jin *et al.*, 1998)	+	-	-	-	-	+	-	-
Frequency	**12/15**	**11/15**	**8/15**	**8/15**	**5/15**	**3/15**	**3/15**	**3/15**
	80%	**73.33 %**	**53.33 %**	**53.33 %**	**33.33 %**	**20%**	**20.00 %**	**20.00 %**

PE, phosphatidylethanolamine; **DPG**, diphosphatidylglycerol; **PI**, phosphatidylinositol; **PG,** phosphatidylglycerol; **HPE,** hydroxyphosphatidylethanolamine**; PC**, phosphatidylcholine; **PME,** phosphatidylmonomethyl-ethanolamine; **LPE**, lysophosphatidylethanolamine.

Goodfellow *et al.* (2012) has observed that, *S. xinjiangensis* exhibit a type PIV phospholipid pattern, because it contains phosphatidylcholine and glucosamine-containing phospholipid, in addition to phosphatidylethanolamine. Even though *S. piscinae*, and *S. xiaoerkulensis* also contain some phosphatidylcholine, but they lack glucosamine-containing phospholipid, to be considered as type PIV. The rest of the phospholipid are variably present as shown in tables 13.

Table 13. Phospholipid content analysis of *Saccharomonosporae*.

Species	PIM	UN PL	UN PGL	UN GPL	LPG	NPG	AL	AP
S. amisosensis (Veyisoglu *et al.*, 2013)	+	-	-	-	-	-	+	+
S. azurea (Klenk *et al.*, 2012)	-	-	-	-	-	-	-	-
S. colocasiae (Wattanasuepsin *et al.*, 2017)	-	-	-	-	-	-	-	-
S. cyanea (Runmao *et al.*, 1988)	-	-	-	-	-	-	-	-
S. glauca (Greiner-Mai *et al.*, 1988)	-	-	-	-	-	-	-	-
S. halophila (Al-Zarban *et al.*, 2002)(Tang *et al.*, 2011)	-	+	-	-	-	-	-	-
S. iraqiensis subsp. *iraqiensis* (Ruan *et al.*, 1994)(Tang *et al.*, 2011)	-	-	-	-	+	-	-	-
S. iraqiensis subsp. *paurometabolica* (Li *et al.*, 2003)	-	-	-	-	-	-	-	-
S. marina (Lieu *et al.*, 2010)	-	-	-	-	-	-	-	-
S. oceani (Zhang *et al.*, 2013)	+	-	+	-	-	-	-	-
S. piscinae (Tseng *et al.*, 2018)	-	-	+	-	-	-	-	-
S. saliphila (Syed *et al.*, 2008)	-	+	-	-	-	+	-	-
S. viridis (Embley *et al.*, 1988)	-	+	+	-	-	-	-	-
S. xiaoerkulensis (Li *et al.*, 2016)	-	+	-	-	-	-	-	-
S. xinjiangensis (Jin *et al.*, 1998)	-	-	-	+	-	-	-	-
Frequency	**2/15**	**3/15**	**3/15**	**1/15**	**1/15**	**1/15**	**1/15**	**1/15**
	13.33 %	**20.00 %**	**20.00 %**	**6.66 %**	**6.66 %**	**6.66 %**	**6.66 %**	**6.66 %**

PIM, phosphatidylinositol-mannoside; **UN PL,** unknown phospholipid; **UN PGL,** unkown phosphoglycolypid; **UN GPL,** unknown glucosamine-containing phospholipid; **LPG,** lysophosphatidylglycerol; **NPG,** ninhydrin-positive phosphoglycolipid; **AL,** aminolipid; **AP,** aminophosphate.

3.1.5 Fatty acid analysis

The fatty acid analysis results (tables in Annex I) show that the lipid profile is highly variable, and there is no common and characteristic fatty acids among all the studied species. However, there is a clear presence of branched iso, and anteiso fatty acid, iso-$C_{16:0}$ (93%), iso-$C_{17:0}$ (86.66%), iso-$C_{15:0}$ (86.66%), anteiso-$C_{17:0}$ (80%), iso-$C_{18:0}$ (46.66%). Saturated and unsaturated fatty acids are less present, such as $C_{16:0}$ (73.33%), $C_{17:0}$ (46.66%), $C_{17:1}$ *w*6*c* (46.66%), $C_{17:1}$ *w*8c (40%). This mixture is generally in accordance with the lipid profile of *Saccharomonospora* as mentioned by Goodfellow *et al.* (2012), which corresponds to lipid type "3a" as defined by Kroppenstedt (1985).

From a taxonomic point of view, it seems that the lipid profile has a little importance in the description of *Sacchomonospora*, at the genus, and subgenus level, in comparison to other cellular, membrane, and cell-wall components, as observed in the Bergey's manual of the phylum of *Actinobacteria* (2012). In addition lipids content ratio can be influenced subtly and unpredictably according to culture media, and culture conditions (temperature, pH and hydrostatic pressure) to maintain the homeostasis in a process called 'homeoviscous adaptation', which is observed in mesophilic and thermophilic bacteria, by increasing the ratio of saturated, and branched iso-fatty acids among other mechanisms (Embley *et al.*, 1988; Patel *et al.* 1991; and Siliakus *et al.,* 2017), this can be further investigated in the genus of *Saccharomnospora* to characterise mesophilic and thermophilic species.

3.2 Similarity based on *Jaccard*'s and *Kulczynski-2*'s coefficients

The chemotaxonomic similarity results according to *Jaccard*'s, and *Kulczynski-2*'s coefficients, has given different similarity order and degree.

The similarity order according to the coefficient of *Jaccard* (table 14) is as follow: *S. piscinae* (PI) > *S. oceani* (OC) > *S. xiaoerkulensis* (XO) > *S. halophila* (HA) > *S. marina* (MA) > S. *iraqiensis* subsp. *iraqiensis* (II) > *S. saliphila* (SA) > *S. colocasiae* (CO) > *S. viridis* (VI) > *S. iraqiensis* subsp. *paurometabolica* (IP) > *S.*

glauca (GL) > *S. xinjiangensis* (XA) > *S. azurea* (AZ) > *S. amisosensis* (AM) > *S. cyanea* (CY).

Table 14. Similarity based on the coefficient of *Jaccard*.

Pair of species	M_{11}	M_{01}	M_{10}	M_{00}	***Jaccard-Sneath*** similarity $J = M_{11} / (M_{11} + M_{01} + M_{10})$	Percentage
S. piscinae/S. piscinae	31	0	0	55	31/31 = 1	100%
S. piscinae/S. oceani	22	9	9	46	22/22+9+9 = 0.55	55%
S. piscinae/S. xiaoerkulensis	19	6	12	49	19/19+6+12 = 0.513	51.3%
S. piscinae/S. halophila	23	22	8	33	23/23+22+8 = 0.433	43.3%
S. piscinae/S. marina	19	15	12	45	19/19+15+12 = 0.413	41.3%
S. piscinae/S. iraqiensis subsp. *iraqiensis*	16	11	15	44	16/16+11+15 = 0.38	38%
S. piscinae/S. saliphila	12	2	19	53	12/12+2+19 = 0.363	36.3%
S. piscinae/S. colocasiae	14	8	17	47	14/14+8+17 = 0.358	35.8%
S. piscinae/S. viridis	15	14	16	41	15/15+14+16 = 0.333	33.3%
S. piscinae/S. iraqiensis subsp. *paurometabolica*	13	9	18	46	13/13+9+18 = 0.325	32.5%
S. piscinae/S. glauca	13	11	18	44	13/13+11+18 = 0.309	30.9%
S. piscinae/S. xinjiangensis	11	10	2	45	11/11+10+20 = 0.268	26.8%
S. piscinae/S. azurea	13	18	18	37	13/13+18+18 = 0.265	26.5%
S. piscinae/S. amisosensis	14	22	17	33	14/14+22+17 = 0.264	26.4%
S. piscinae/S. cyanea	9	11	22	44	9/9+11+22= 0.214	21.4%

The similarity order according to *Kulczynski-2* coefficient (table 15) is as follow: PI> OC> XO> HA> SA> MA> II> CO> IP> VI> GL> XA> AM> AZ> CY. The order of the first is the same, with few interchanging position in the middle and the end.

It is expected to find different similarity values and order, when using different similarity coefficients. Even though, both *Jaccard*'s and *Kulczynski-2*'s coefficients share the disregarding of the joint absence of a feature (Albuquerque *et al.*, 2022). However, the clustering data sets will give another view of the kinship.

Table 15. Similarity based on the coefficient of *Kulczynski-2*.

Pair of species	M_{11}	M_{01}	M_{10}	M_{00}	***Kulczynski-2*** similarity $J = [(M_{11} / M_{11} + M_{01}) + (M_{11} / M_{11} + M_{10})]/2$	Percentage
S. piscinae/S. piscinae	31	0	0	55	[(31/31+0) + (31/31+0)]/2 = 1	100%
S. piscinae/S. oceani	22	9	9	46	[(22/22+9) + (22/22+9)]/2 = 0,71	71%
S. piscinae/S. xiaoerkulensis	19	6	12	49	[(19/19+6) + (19/19+12)]/2 = 0,686	68.6%
S. piscinae/S. halophila	23	22	8	33	[(23/23+22) + (23/23+8)]/2 = 0,627	62.7%

Table 14. –Continued- Similarity based on the coefficient of *Kulczynski-2*.

S. piscinae/S. saliphila	12	2	19	53	[(12/12+2) + (12/12+19)]/2 = 0,622	62.2%
S. piscinae/S. marina	19	15	12	45	[(19/19+15) + (19/19+12)]/2 = 0,586	58.6%
S. piscinae/S. iraqiensis subsp. *iraqiensis*	16	11	15	44	[(16/16+11) + (16/16+15)]/2 = 0,554	55.4%
S. piscinae/S. colocasiae	14	8	17	47	[(14/14+8) + (14/14+8)]/2 = 0,544	54.4%
S. piscinae/S. iraqiensis subsp. *paurometabolica*	13	9	18	46	[(13/13+9) + (13/13+18)]/2 = 0,505	50.5%
S. piscinae/S. viridis	15	14	16	41	[(15/15+14) + (15/15+16)]/2 = 0,501	50.1%
S. piscinae/S. glauca	13	11	18	44	[(13/13+11) + (13/13+18)]/2 = 0,481	48.1%
S. piscinae/S. xinjiangensis	11	10	20	45	[(11/11+10) + (11/11+20)]/2 = 0,439	43.9%
S. piscinae/S. amisosensis	14	22	17	33	[(14/14+22) + (14/14+17)]/2 = 0,41	42%
S. piscinae/S. azurea	13	18	18	37	[(13/13+18) + (13/13+18)]/2 = 0,419	41.9%
S. piscinae/S. cyanea	9	11	22	44	[(9/9+11) + (9/9+22)]/2 = 0,37	37%

The *PAST 4* is used to construct Agglomerative Hierarchical Clustering dendograms (AHC) from the chemotaxonomic data based on the two coefficients, using the Neighbour Joining (NJ) clustering method.

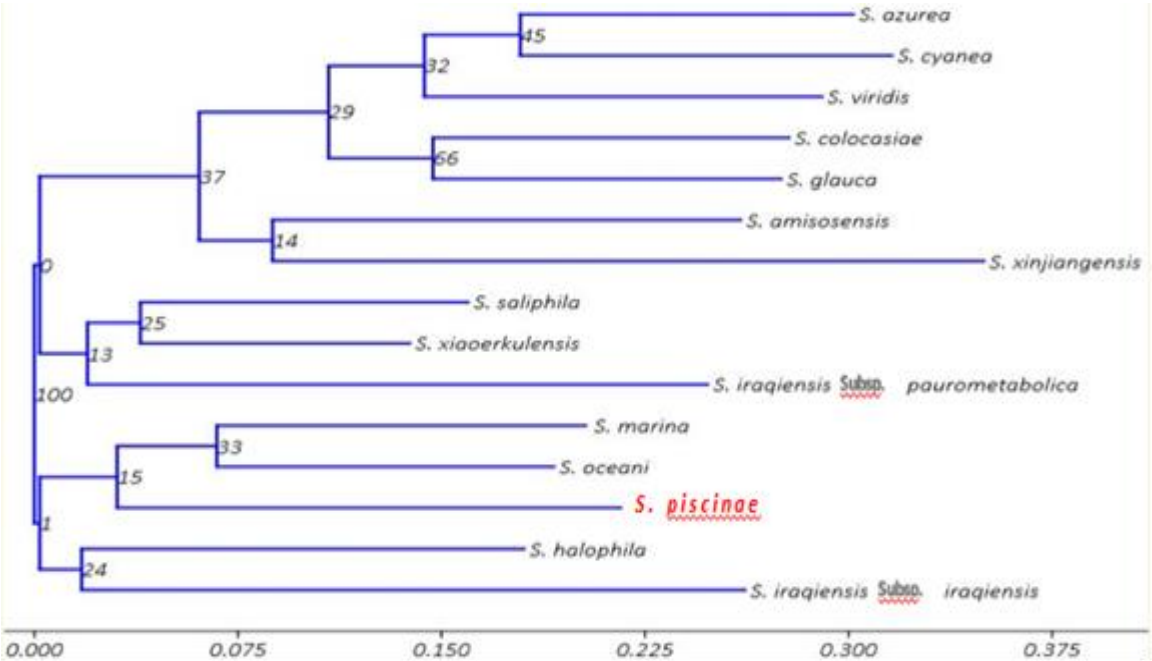


Figure 14. Chemotaxonomy dendogram based on *Kulczynski-2*'s coefficient, using Neighbour Joining method.

The dendogram in figure 14 that is based on *Jaccard*'s coefficient shows that we can classify the 15 species and subspecies into 3 distinct clades. Clades A and B, that are set apart, which contain *S. halophila* and *S. iraqiensis* subsp. *iraqiensis* for clade A, and *S. piscinae* and *S. oceani* for clade B. The large Clade C, which can be in turn divided into 2 subclades which also can be divided further into 2 more subclades each, which contains the rest of the species.

The dendogram in figure 15, that is based on *Kulczynski-2* coefficient; shows different topology. Two major clades that can be divides further into more subclades.

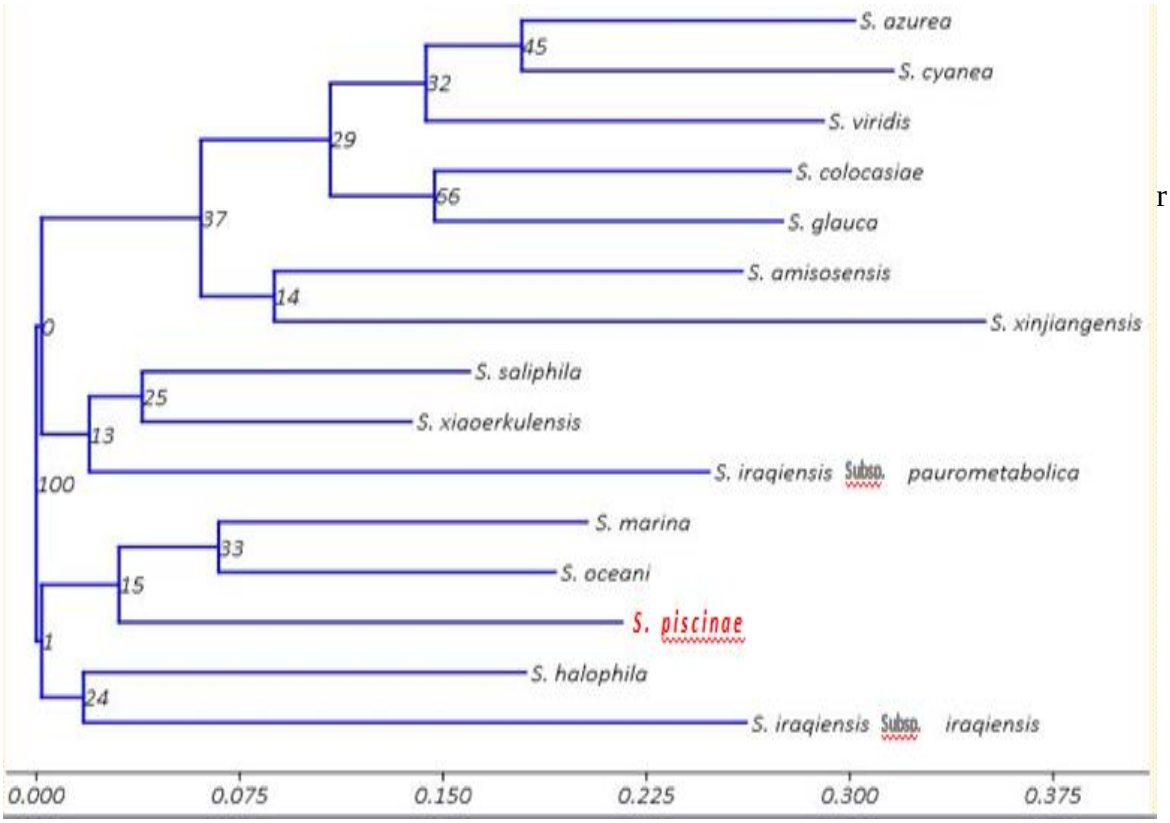


Figure 16. Chemotaxonomy dendogram based on *Kulczynski-2*'s coefficient, using Neighbour Joining method.

Clades A, and B in the previous dendogram are joined together in one superclade, but still separate, and contain the same species, except for *S. marina* that joins *S. oceani* in one subclade that form with *S. piscinae* the formerly clade B in figure 14. The other superclade can be divided into another 2 subclades and each one of them can be divided further into two more subclades.

3.3 Molecular analysis

After multiple alignment of the 16S rRNA gene sequences of the 15 species and subspecies of the genus *Saccharomonospora* using ClustalW (Thompson *et al.*, 1994), a phylogenetic tree (figure 16) was constructed by the N J method (Saitou and Nei, 1987).

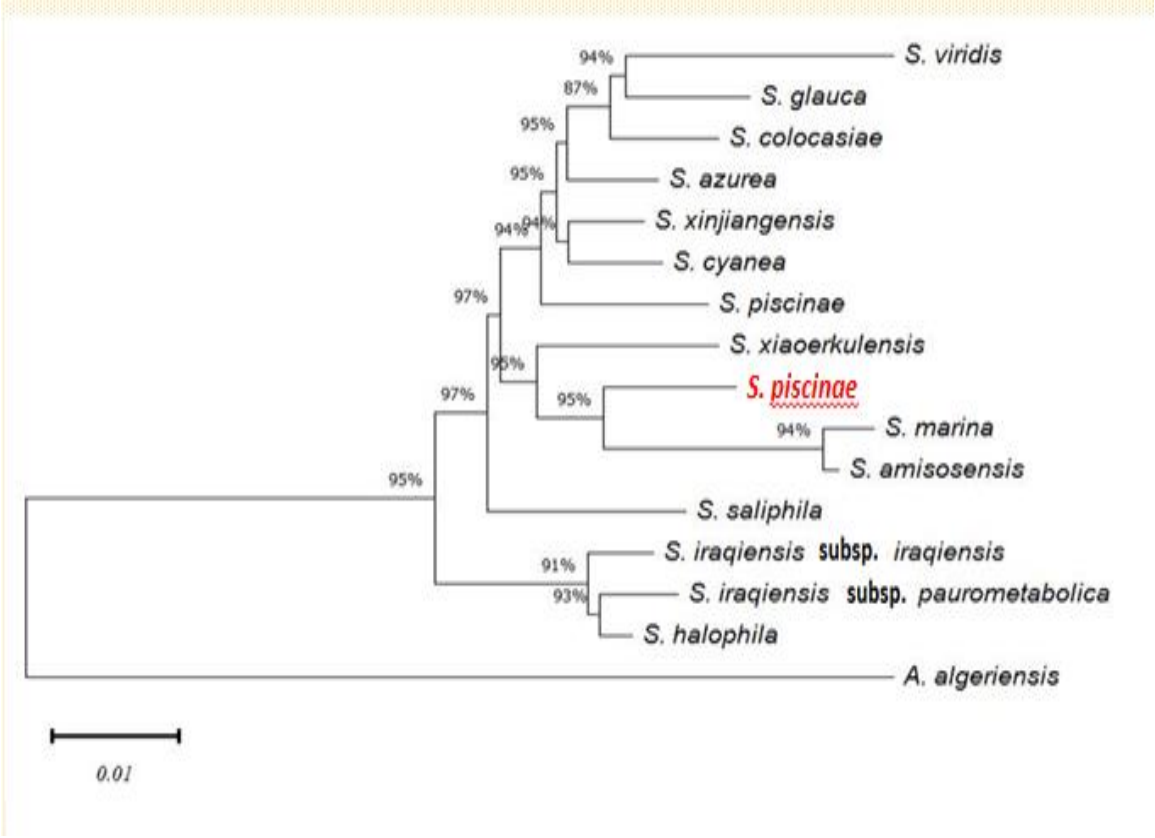


Figure 17. 16S rRNA phylogeny tree using Neighbour Joining method.

The topology of the phylogenetic tree is consistent with the topology of the 16S rRNA gene phylogenetic tree of the works of Meier-Kolthoff *et al.* (2013), Nouioui *et al.* (2018), and Ramírez-Durán *et al.* (2021). Conversely, it resulted in a completely different tree topology compared to the chemotaxonomic approach. The evolution distances can be calculated using the branches length of the tree, which are presented in table 17, and the similarity order is set and compared to the chemotaxonomic and EZbiocloud results.

The results of the similarity research from EZbiocloud are represented in table 16, with the sequencing completeness of the 16S rRNA gene, and the number of

mismatches compared to *S. piscinae* (Yoon *et al*., 2017). The similarity order in relation to *S. piscinae*, is given as follow: *S. azurea* (98.27%), *S. xinjiangensis* (97.99%), *S. cyanea* (97.65%), *S. colocasiae* (97.53%), *S. glauca* (97.44), *S. saliphila* (97.17%), *S. oceani* (97.02%), *S. xiaoerkulensis* (96.96%), *S. iraqiensis* subsp. *paurometabolica* (96.53%), *S. amisosensis* (96.14%), *S. marina* (96.14%), *S. iraqiensis* subsp. *iraqiensis* (96.06%), *S. halophila* (95.92%), and *S. viridis* (95.78%).

Table 16. Similarity order according to EZbiocloud.

Name	Pairwise Similarity (%)	Mismatch/Total Nt	Completeness (%)
S. piscinae	100	0/1451	100
S. azurea	98.27	25/1447	100
S. xinjiangensis	97.99	29/1445	100
S. cyanea	97.65	34/1445	100
S. colocasiae	97.53	34/1376	95.43
S. glauca	97.44	37/1446	100
S. saliphila	97.17	41/1449	100
S. oceani	97.02	43/1443	99.72
S. xiaoerkulensis	96.96	44/1448	100
S. iraqiensis subsp. *paurometabolica*	96.53	50/1443	100
S. amisosensis	96.14	56/1449	100
S. marina	96.13	56/1447	100
S. iraqiensis subsp. *iraqiensis*	96.06	57/1445	100
S. halophila	95.92	59/1445	100
S. viridis	95.78	61/1445	100

Nt, nucleotide

In table 17, a comparison of similarity order between the studied species and subspecies of the genus *Saccharomonospora*, was carried out, and we have noticed that chemotaxonomy based on *Jaccard*'s coefficient has given a completely identical similarity order with the 16S rRNA phylogeny using NJ method, but a major difference in similarity order to both EZbioloud phylogeny, and *Kulczynski-2*'s coefficient chemotaxonomy. The order of similarity in the 16S rRNA phylogeny using the NJ method, is calculated from the branches length of the phylogeny tree, and are set in the increasing order of the evolution distances.

The two phenetic trees based on the *Jaccard*'s coefficient of association, and the phylogenetic tree based on 16S rRNA gene sequences using the same clustering

method (NJ) has a given a strong indication that *S. viridis, S. glauca, S. colocasiae, S. azurea*, and *S. cyanea* indeed belong to the same subclade as confirmed by the three methods, which is in concordance with the study of Pat *et al.* (2009) that used Maximum Likelihood method. The same topology has been confirmed with the phylogenomic study of Ramírez-Durán *et al.* (2021). However, the three methods fails to show that *S. iraqiensis* subsp. *iraqiensis* and *S. iraqiensis* subsp. *paurometabolica* belong to the same species, even though, the 16S rRNA phylogeny based on NJ showed a close kinship between the 2 subspecies of *S. iraqiensis* and *S. halophila*. This unstable taxonomic position was further investigated and settled by dDDH, in favour of considering *S. iraqiensis* formerly classified as *Actinopolyspora iraqiensis* (Ruan *et al.*, 1994), and *S. paurometabolica* (Li *et al.*, 2003), as subspecies of *S. iraqiensis* (Nouinoui *et al.*, 2018), even though Ramírez-Durán (2021) has suggested that *S. halophila* could be another subspecies of *S. iraqiensis*.

It is noteworthy, that there was no difference in the topology of the phylogenic tree using NJ, and MP (Maximum Parsimony) methods. However a significant difference is noticed between the similarity order of the studied species and subspecies compared to *S. piscinae* using *MEGA 11* based on NJ method, and the similarity order based on EZbiocloud, which could be due to higher 16S rRNA sequences quality and the use of different phylogenic method (Chum *et al.*, 2007).

Table 17. Similarity comparison between chemotaxonomy (*Jaccard*) and the molecular taxonomy approach.

Order of similarity in relation to *S. piscinae*	**Chemotaxonomy**	**Molecular Phylogeny**	
	Jaccard	**N J**	**EZbiocloud**
S. piscinae	100	0	100
S. oceani	55	0.0193	97.02
S. xiaoerkulensis	51.35	0.0195	96.96
S. halophila	43.39	0.0207	95.92
S. saliphila	41.30	0.025	97.17
S. marina	38.09	0.0272	96.13
S. iraqiensis subsp. *iraqiensis*	36.36	0.0305	96.06
S. colocasiae	35.89	0.0306	97.53
S. iraqiensis subsp. *paurometabolica*	33.33	0.0334	96.53

Table 17. –Continued- Similarity comparison between chemotaxonomy (*Jaccard*) and the molecular taxonomy approach

S. viridis	32.50	0.0341	95.78
S. glauca	30.95	0.0377	97.44
S. xinjiangensis	26.82	0.0394	97.99
S. amisosensis	26.53	0.04	96.14
S. azurea	26.41	0.042	98.27
S. cyanea	21.42	0.1186	97.65
Order of similarity	PI/PI-OC-XO-HA-SA-MA-II-CO-IP-VI-GL-XA-AM-AZ-CY		PI/PI-AZ-XI-CY-CO-GL-SA-OC-XO-IP-AM-MA-II-HA-VI

S. piscinae **(PI)**; *S. oceani* **(OC)**; *S. xiaoerkulensis* **(XO)**; *S. halophila* **(HA)**; *S. marina* **(MA)**; S. *iraqiensis* subsp. *iraqiensis* **(II)**; *S. saliphila* **(SA)**; *S. colocasiae* **(CO)**; *S. viridis* **(VI)**; *S. iraqiensis* subsp. *paurometabolica* **(IP)**; *S. glauca* **(GL)**; *S. xinjiangensis* **(XA)**; *S. azurea* **(AZ)**; *S. amisosensis* **(AM)**; *S. cyanea* **(CY); N J**, Neihbour Joining.

Even though the chemotaxonomy analysis, is of major importance for *Saccharomonospora* at the genus level (Goodfellow *el al.*, 2012), it has been shown to be unsatisfactory to discriminate at the species level as confirmed by Greiner-mai *et al*. (1988), even with the presence of characteristic features in some species, such as in the case of phosphatidylcholine and glucosamine-containing phospholipid in S. *xinjiangensis*, and the presence of MK-10(H_4) and LL-DAP amino acid in *S. iraqiensis* subsp. *iraqiensis*, and the presence of N-acetylmuramic acid in *S. colocasiae*, and the presence of madurose in *S. piscenae*, and the presence of xylose in *S. azurea*, and finally the absence of teichoic acid in *S. viridis*.

The chemotaxonomy also suffers from a lack of information due to the ever increasing reliance on the molecular approaches (Kim, 2015), which makes it inconclusive, as is the case for lack of information about the polar lipid profile for *S. azurea* (Klenk *et al.*, 2012), and *S. cyanea* (Runmao *et al.*, 1988), and also the unclear results if some analysis the report unkown sugars *S. halophila* (Al-Zarban *et al.*, 2002), and lipids (Zhang *et al.*, 2013). All this can affect the chemotaxonomy results severely.

Based on the deficiencies of the chemotaxonomy stated above, in addition to the remark of Embley *et al*. (1994) and Stackebrandt and Schumann (2006), that the huge diversity in the phylum of *Actinobacteria* cannot guarantee a consistency in the chemotaxonomic features among different taxa. For these reasons we take reservedly the results of the numerical chemotaxonomy based on the coefficient of *Jaccard*, and the coefficient of *Kuclzynski-2*, of the 15 species and subspecies of

Saccharomonospora, even with the surprising correspondence between the similarity order given be *Jaccard* coefficient and 16S rRNA phylogeny using the N J method.

Even with the critics that have been described for the 16S rRNA phylogeny for some genera, it has proven so far to be a true reflection of the whole genome phylogeny. Ramírez-Durán *et al.*(2021) has recently confirmed that 16S rRNA gene-based phylogenies is still pretty much reliable in the taxonomy of *Saccharomonosporae*, with no significant differences in topology between the phylogenetic and the phylogenomic trees.


Conclusion &
perspectives

Nonomura and Ohara (1971) has proposed and created the genus *Saccharomonospora* for type IV cell wall monosporic actinomycetes. Subsequently Kroppenstedt (1985) further elaborated the scope of the biochemical features of this genus. It is well set that the morphology description at both macro and micro level is a good indicator of the genus *Saccharomonspora*. However, the classification of this genus at the species level proved to be ambiguous and less certain (Goodfellow and Pirouz, 1982; McCarthy and Cross, 1984), and the genus *Saccharomonospora* was coined as a "taxonomically troubled genus with bioenergetic potential" (Klenk *et al.*, 2012; Meier-Kolthoff *et al.*, 2013).

The primary objective of this study is to test whether the chemotaxonomy alone could be a reliable method for differentiation and discrimination between *Saccharomonosporae*, and the way to prove this is to compare it with the molecular approach based on the similarity and phylogeny using 16S rRNA gene sequences, which has massively impacted the taxonomy of bacteria at many taxonomic levels (Rossi-Tamisier *et al.*, 2015).

Despite the results that show a consistency between chemotaxonomy analysis based on *Jaccard* coefficient in one hand, and the 16S rRNA molecular approach based on Neighbour Joining method in the other hand, these results are best taken prudently, because of limited available chemotaxonomic data in general, and of some species of this genus in particular. For thess reasons, this comparative study should be applied to more thoroughly chemically studied genera, and should take in consideration a larger group to insure statistical significance. Future works should also extend the numerical study to more statistical coefficients for more consistency. Moreover, it is wise to suggest not relying only on the Neighbour-Joining phylogeny method, and the study should take in consideration other methods such as Maximum Parsimony, and Maximum Likelihood.

In addition to the classical polyphasic approache, many other tools for better classification and identification of this genus can be suggested such as the study of the esterase pattern, DNA restriction patterns, protein patterns, phage typing, and taxogenomic and comparative genomic Analysis, with focus on the biosynthetic gene clusters (BGCs): polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS).

Bibliographic references

Abbas, I. H. (2006). Biological and Biochemical Studies of actinomycetes isolated from Kuwait Saline Soil-Kuwait. *Journal of applied sciences research*, 2 (10), 809-815.

Albuquerque, M. A. de, Nascimento, E. R. do., Barros, K. N. N. de O., & Barros, P. S. N. (2022). Comparison between similarity coefficients with application in forest sciences. *Research, Society and Development*, *11*(2), e48511226046.

Alderson, G., Goodfellow, M., & Minnikin, D. E. (1985). Menaquinone composition in the classification of *Streptomyces* and other sporoactinomycetes. *Microbiology*, 131(7), 1671-1679.

Al-Mahdi, A.Y. (2016). New Records of *Streptomyces* and Non *Streptomyces* Actinomycetes Isolated from Soils Surrounding Sana'a High Mountain. *International Journal of Research in Pharmacy and Biosciences*. 3(3): 19-31.

Alshaibani, M. (2021). Actinobacteria and Environmental Adaptations. In *Encyclopedia*. https://encyclopedia.pub/entry/12818.

Al-Zarban, S. S., Al-Musallam, A. A., Abbas, I., Stackebrandt, E., & Kroppenstedt, R. M. (2002b). *Saccharomonospora halophila* sp. nov., a novel halophilic actinomycete isolated from marsh soil in Kuwait. *Int. J. Syst. Evol. Microbiol.* 52, 555–558.

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Meier-Kolthoff, J. P., Klenk, H. P., Clément, C., Ouhdouch, Y., & van Wezel, G. P. (2015). Taxonomy, Physiology, and Natural Products of Actinobacteria. *Microbiology and molecular biology reviews : MMBR*, *80*(1), 1–43.

Becker, B., Lechevalier, M. P., & Lechevalier, H. A. (1965). Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. *Appl. Microbiol.* 13:236–243

Berdy, J. (2005). Bioactive microbial metabolites. *The Journal of antibiotics*, *58*(1), 1-26.

Boudjelal-Bencheikh, F. (2012). Taxonomie et antagonisme des actinomycètes halophiles d'origine saharienne et caractérisation des composés bioactifs sécrétés par *Actinoalloteichus*sp. AH97 (Dissertation doctorale).

Bouras, S., & Fartas, S. (2021). Etude de comparaison chimiotaxonomique (à la base de coefficient de *Jaccard*) et moléculaire du genre *Actinoalloteichus*. MSc. Université de Ghardaia.

Busse, H. J., Denner, E. B., & Lubitz, W. (1996). Classification and identification of bacteria: current approaches to an old problem. Overview of methods used in bacterial systematics. *Journal of biotechnology*, *47*(1), 3–38.

Cheetham, A. H., & Hazel, J. E. (1969). Binary (Presence-Absence) Similarity Coefficients. *Journal of Paleontology*, *43*(5), 1130–1136.

Chen, X., Jiang, Y., Li, Q., Han, L., & Jiang, C. (2016). Molecular Phylogenetic Identification of *Actinobacteria*. *InTech*.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* 57, 2259-2261.

Collins, M. D., & Jones, D. (1981). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiological reviews*, *45*(2), 316–354.

Collins, MD., Pirouz, T., Goodfellow, M., & Minnikin, DE. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol.*, 100:221–230.

Collins, M. D., Goodfellow, M., Minnikin, D. E., & Alderson, G. (1985). Menaquinone composition of mycolic acid-containing actinomycetes and some sporoactinomycetes, *Journal of applied bacteriology*, 58(1), 77-86.

Cundell, A. M., & Piechoski, J. T. (2016). Actinomycetes. In Encyclopedia of Food Microbiology (pp. 1-7). Academic Press.

Cunha, F., Sousa, D. L., Trindade, L., & Duque, V. (2022). Disseminated cutaneous *Actinomyces bovis* infection in an immunocompromised host: case report and review of the literature. *BMC infectious diseases*, *22*(1), 310.

Embley, M.T., Smida, J., & Stackebrandt, E. (1988). The Phylogeny of Mycolate-less Wall Chemotype IV Actinomycetes and Description of *Pseudonocardiaceae* fam. nov. *Systematic and Applied Microbiology, 11*, 44-52.

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

Goel, N.; Fatima, S.W.; Kumar, S.; Sinha, R. & Khare, S.K. (2021). Antimicrobial resistance in biofilms: Exploring marine actinobacteria as a potential source of antibiotics and biofilm inhibitors. Biotechnology Reports (Amsterdam, Netherlands). 30: e00613.

Goodfellow, M. & Williams, S.T. (1983) Ecology of Actinomycetes. *Annual Review of Microbiology*, 37, 189-216.

Goodfellow, M., & Minnikin, D. E. (1985). *Chemical methods in bacterial systematics* (No. 20). Academic Press.

Goodfellow, M., & Pirouz, T. (1982). Numerical classification of sporoactinomycetes containing meso-diaminopimelic acid in the cell wall. *Microbiology*, *128*(3), 503-527.

Goodfellow, M., Peter, K. Ã., Hans-JÃrgen Busse, Trujillo, M. E., Ludwig, W., Suzuki, K. I., & Parte, A. (2012). Bergey's manual of systematic bacteriology: Volume 5: *The Actinobacteria*. New York, NY: Springer New York.

Greiner-mai, E., Korn-wendisch, F., & Kutzner, H. J. (1988). Taxonomic Revision of the Genus *Saccharomonospora* and Description of *Saccharomonospora glauca* sp. nov. *Int. J. Syst. Evol. Microbiol., 38*(4), 398-405.

Hammer, Ø., Harper, D.A.T., & Ryan, P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 9pp.

Ismail, S., Jiang, B., Nasimi, Z., Inam-Ul-Haq, M., Yamamoto, N., Danso Ofori, A., Khan, N., Arshad, M., Abbas, K., & Zheng, A. (2020). Investigation of *Streptomycesscabies* Causing Potato Scab by Various Detection Techniques, Its Pathogenicity and Determination of Host-Disease Resistance in Potato Germplasm. *Pathogens* (Basel, Switzerland), *9*(9), 760.

Jaccard, P. (1912). The distribution of the flora in the Alpine zone.. New Phytologist, 11: 37-50.

Jagannathan, S.V., Manemann, E.M., Rowe, S.E., Callender, M.C., & Soto, W. (2021). Marine Actinomycetes, New Sources of Biotechnological Products. Marine Drugs. 19(7): 365.

Jin, X., Xu, L. H., Mao, P. H., Hseu, T. H., & Jiang, C. L. (1998). Description of *Saccharomonospora xinjiangensis* sp. nov. based on chemical and molecular classification. *International journal of systematic bacteriology*, *48 Pt 4*, 1095–1099.

Kenneth, H. Wilson. (1995). Molecular Biology as a Tool for Taxonomy. *Clinical Infectious Diseases*, *20*, S117–S121. http://www.jstor.org/stable/4458632

Kim, M., Oh, H. S., Park, S. C., & Chun, J. (2014). Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int. J. Syst. Evol. Microbiol.*, *64*(Pt_2), 346-351.

Kim, M. & Chun, J. (2014). 16S rRNA Gene-Based Identification of Bacteria and Archaea using the EzTaxon Server.

Kim, S. B. (2015). *Saccharomonospora* in *Bergey's Manual of Systematics of Archaea and Bacteria.* Chichester, UK: John Wiley & Sons, Ltd, 1–11.

Kim, S. B., Yoon, J. H., Kim, H., Lee, S. T., Park, Y. H., & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Saccharomonospora* conducted with 16S rRNA gene sequences. *International journal of systematic bacteriology*, *45*(2), 351–356.

Klenk, H. P., Lu, M., Lucas, S., Lapidus, A., Copeland, A., Pitluck, S., Goodwin, L. A., Han, C., Tapia, R., Brambilla, E. M., Pötter, G., Land, M., Ivanova, N., Rohde, M., Göker, M., Detter, J. C., Li, W. J., Kyrpides, N. C., & Woyke, T. (2012). Genome sequence of the ocean sediment bacterium *Saccharomonospora marina* type strain (XMU15(T)). *Standards in genomic sciences*, *6*(2), 265–275.

Klenk, H. P., Held, B., Lucas, S., Lapidus, A., Copeland, A., Hammon, N., Pitluck, S., Goodwin, L. A., Han, C., Tapia, R., Brambilla, E. M., Pötter, G., Land, M., Ivanova, N., Rohde, M., Göker, M., Detter, J. C., Kyrpides, N. C., & Woyke, T. (2012). Genome sequence of the soil bacterium *Saccharomonospora azurea* type strain (NA-128(T)). *Standards in genomic sciences*, *6*(2), 220–229.

Klenk, H. P., Held, B., Lucas, S., Lapidus, A., Copeland, A., Hammon, N., Pitluck, S., Goodwin, L. A., Han, C., Tapia, R., Brambilla, E. M., Pötter, G., Land, M., Ivanova, N., Rohde, M., Göker, M., Detter, J. C., Kyrpides, N. C., & Woyke, T. (2012). Genome sequence of the soil bacterium *Saccharomonospora azurea* type strain (NA-128(T)). *Standards in genomic sciences*, *6*(2), 220–229.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution, 38*, 3022 - 3027.

Krieg, N. R., & Padgett, P. J. (2011). Phenotypic and physiological characterization methods. In *Methods in microbiology*, 38: 15-60. Academic Press.

Kroppenstedt, R. E. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. *Chemical Methods in Bacterial Systematics*, 173-199.

Kroppenstedt, R. M., & Evtushenko, L. I. (2006). The family *Nocardiopsaceae*. *The Prokaryotes: A Handbook on the Biology of Bacteria,, 3*, 754-795.

Kroppenstedt, R. M., Korn-Wendisch, F., Fowler, V. J., & Stackebrandt, E. (1981). Biochemical and molecular genetic evidence for a transfer of *Actinoplanes armeniacus* into the family *Streptomycetaceae*. *Zentralblatt für Bakteriologie Mikrobiologie und Hygiene: I. Abt. Originale C: Allgemeine, angewandte und ökologische Mikrobiologie*, *2*(3), 254-262.

Labeda, D. P., & Lechevalier, M. P. (1989). Amendment of the genus *Saccharothrix* Labeda *et al*. 1984 and descriptions of *Saccharothrix espanaensis* sp. nov., *Saccharothrix cryophilis* sp. nov., and *Saccharothrix mutabilis* comb. nov. *Int. J. Syst. Bacteriol.*, 39 (4): 420-423.

Larpent, J. P. (2000). Introduction à la nouvelle classification bactérienne: les principaux groupes bactériens. Tec et Doc. France.

Lechevalier, M. P., & Lechevalier, H. A. (1965). Classification des actinomycètes aérobies basée sur leur morphologie et leur composition chimique. Ann Inst Pasteur; 108:662–73.

Lechevalier, M. P. (1968). Identification of aerobic actinomycetes of clinical importance. *The Journal of laboratory and clinical medicine*, *71*(6), 934-944.

Lechevalier, M. P., & Lechevalier, H. A. (1970). Composition of whole-cell hydrolysates as a criterion in the classification of aerobic actinomycetes. In: *The Actinomycetales*. Prauser H. (Eds.) G. Fisher Verlag, Jena. pp. 311-316.

Lechevalier, M. P., & Lechevalier, H. A. (1980). Lipid composition in the classification of *Nocardiae* and *Mycobacteria*. *J Bacteriol*.;105:313–8.

Lechevalier, M. P., & Lechevalier, H. A. (1980). The chemotaxonomy of actinomycetes, p 225–292. *In* Dietz A, Thayer DW (ed), Actinomycetes taxonomy, vol A6 Virginia Society of Industrial Microbiology, Arlington, VA.

Lechevalier, M. P., de Bièvre, C. & Lechevalier H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem. Syst. Ecol*., 5 (4): 249-260.

Legendre, P. and Legendre, L. (1998) Numerical ecology. 2nd English Edition, Elsevier, Amsterdam.

Li, D., Chen, Z. J., Luo, X. X., Xia, Z. F., Wan, C. X., & Zhang, L. L. (2016). *Saccharomonospora xiaoerkulensis* sp. nov., isolated from lake sediment. *Int. J. Syst. Evol. Microbiol.*, *66*(12), 5145–5149.

Li, Q., Chen, X., Jiang, Y., & Jiang, C. (2016). Morphological identification of *Actinobacteria*. *Actinobacteria-basics and biotechnological applications*, 59-86.

Li, Q., Chen, X., Jiang, Y., & Jiang, C. (2016). Cultural, Physiological, and Biochemical Identification of *Actinobacteria*. InTech.

Li, W. J., Tang, S. K., Stackebrandt, E., Kroppenstedt, R. M., Schumann, P., Xu, L. H., & Jiang, C. L. (2003). *Saccharomonospora paurometabolica* sp. nov., a moderately halophilic actinomycete isolated from soil in China. *Int. J. Syst. Evol. Microbiol.*, *53*(Pt 5), 1591–1594.

Liu, Z., Li, Y., Zheng, L. Q., Huang, Y. J., & Li, W. J. (2010). *Saccharomonospora marina* sp. nov., isolated from an ocean sediment of the East China Sea. *Int. J. Syst. Evol. Microbiol., 60*(Pt 8), 1854–1857.

Ludwig, W., & Klenk, H. P. (2005). Overview: a phylogenetic backbone and taxonomic framework for procaryotic systematics. *Bergey's manual® of systematic bacteriology*, 49-66.

Ludwig, W., Euzéby, J., Schumann, P., Busse, H. J., Trujillo, M. E., Kämpfer, P., & Whitman, W. B. (2012). Road map of the phylum *Actinobacteria*. In *Bergey's manual® of systematic bacteriology* (pp. 1-28). Springer, New York, NY.

McCarthy, A. J., & Cross, T. (1984). A taxonomic study of *Thermomonospora* and other monosporic actinomycetes. *Microbiology, 130*(1), 5-25.

Meier-Kolthoff, J. P., Lu, M., Huntemann, M., Lucas, S., Lapidus, A., Copeland, A., Pitluck, S., Goodwin, L. A., Han, C., Tapia, R., Pötter, G., Land, M., Ivanova, N., Rohde, M., Göker, M., Detter, J. C., Woyke, T., Kyrpides, N. C., & Klenk, H. P. (2013). Genome sequence of the chemoheterotrophic soil bacterium *Saccharomonospora cyanea* type strain (NA-134(T)). *Standards in genomic sciences, 9(1)*, 28–41.

Miyadoh, S. (1997). Atlas of actinomycetes. Society for Actinomycetes Japan.

Nguyen, N. P., Warnow, T., Pop, M., & White, B. (2016). A perspective on 16S rRNA operational taxonomic unit clustering using sequence similarity. *NPJ biofilms and microbiomes, 2*, 16004.

Nonomura, H., & Ohara, Y. (1971). Distribution of actinomycetes in soil. XI. Some new species of. *J. Ferment. Technol., 49*, 904-912.

Nouioui, I., Carro, L., García-López, M., Meier-Kolthoff, JP., Woyke, T., Kyrpides, NC., Pukall, R., Klenk, H-P., Goodfellow, M., & Göker, M. (2018). Genome-Based Taxonomic Classification of the Phylum *Actinobacteria*. Front. Microbiol. 9:2007.

Numoto, N., Kamiya, N., Bekker, G. J., Yamagami, Y., Inaba, S., Ishii, K., Uchiyama, S., Kawai, F., Ito, N., & Oda, M. (2018). Structural Dynamics of the PET-Degrading Cutinase-like Enzyme from *Saccharomonospora viridis* AHK190 in Substrate-Bound States Elucidates the Ca2+-Driven Catalytic Cycle. Biochemistry, 57(36), 5289–5300.

Parte, A. C. (2014). LPSN–list of prokaryotic names with standing in nomenclature. *Nucleic Acids Res* 42 (D1), D613–D616.

Parte, A.C., Sardà Carbasse, J., Meier-Kolthoff, J.P., Reimer, L.C., & Göker, M. (2020). List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int. J. Syst. Evol. Microbiol.*, 70, 5607-5612.

Patel, B. K. C., Skerratt, J. H., & Nichols, P. D. (1991). The phospholipid ester-linked fatty acid composition of thermophilic bacteria. *Systematic and applied microbiology, 14*(4), 311-316.

Pati, A., Sikorski, J., Nolan, M., Lapidus, A., Copeland, A., Glavina Del Rio, T., Lucas, S., Chen, F., Tice, H., Pitluck, S., Cheng, J. F., Chertkov, O., Brettin, T., Han, C., Detter, J. C., Kuske, C., Bruce, D., Goodwin, L., Chain, P., D'haeseleer,

P., & Klenk, H. P. (2009). Complete genome sequence of *Saccharomonospora* viridis type strain (P101). *Standards in genomic sciences*, *1*(2), 141–149.

Patil, P. B., Zeng, Y., Coursey, T., Houston, P., Miller, I., & Chen, S. (2010). Isolation and characterization of a *Nocardiopsis* sp. from honeybee guts. *FEMS microbiology letters*, *312*(2), 110–118.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. Q. (2013). The silva ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41 (Database issue), D590–D596.

Raghu, G., Remy-Jardin, M., Ryerson, C. J., Myers, J. L., Kreuter, M., Vasakova, M., Bargagli, E., Chung, J. H., Collins, B. F., Bendstrup, E., Chami, H. A., Chua, A. T., Corte, T. J., Dalphin, J. C., Danoff, S. K., Diaz-Mendoza, J., Duggal, A., Egashira, R., Ewing, T., Gulati, M., & Wilson, K. C. (2020). Diagnosis of Hypersensitivity Pneumonitis in Adults. An Official ATS/JRS/ALAT Clinical Practice Guideline. *American journal of respiratory and critical care medicine*, *202*(3), e36–e69.

Ramírez-Durán, N., de la Haba, R. R., Vera-Gargallo, B., Sánchez-Porro, C., Alonso-Carmona, S., Sandoval-Trujillo, H., & Ventosa, A. (2021). Taxogenomic and comparative genomic analysis of the genus *Saccharomonospora* focused on the identification of biosynthetic clusters PKS and NRPS. *Frontiers in Microbiology*, *12*, 603791.

Ranjani, A., Dhanasekaran, D., & Gopinath, P. M. (2016). An Introduction to *Actinobacteria*. InTech.

Rossi-Tamisier, M., Benamar, S., Raoult, D., & Fournier, P.-E. (2015). Cautionary tale of using 16S rRNA gene sequence similarity values in identification of human-associated bacterial species. *Int. J. Syst. Evol. Microbiol.*, *65*(Pt_6), 1929-1934.

Ruan, J. S., Lang, Y., Shi, Y., Qu, L., & Yu, X. (1994). Chemical and molecular classification of *Saccharomonospora* strains. *International journal of systematic bacteriology*, *44*(4), 704–707.

Ruan, J.-S., Al-tai, A. M., Zhou, Z.-H., & Qu, L.-H. (1994). *Actinopolyspora iraqiensis* sp. nov., a New Halophilic Actinomycete Isolated from Soil. *Int. J. Syst. Evol. Microbiol. M*, *44*(4), 759-763.

Runmao, H. (1987). *Saccharomonospora azurea* sp. nov., a New Species from Soil. *Int. J. Syst. Evol. Microbiol.*, *37*(1), 60-61.

Runmao, H., LIN, C., & Guizhen, W. (1988). *Saccharomonospora cyanea* sp. nov. *Int. J. Syst. Evol. Microbiol. M*, *38*(4), 444-446.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4:406-425.

Saker, R., Bouras, N., Meklat, A., Zitouni, A., Schumann, P., Spröer, C. & Klenk, H. P. (2015). *Prauserella isguenensis* sp. nov., a halophilic actinomycete isolated from desert soil. *Int. J. Syst. Evol. Microbiol.*, *65*(Pt_5), 1598-1603.

Sayed, A. M., Hassan, M. H. A., Alhadrami, H. A., Hassan, H. M., Goodfellow, M., & Rateb, M. E. (2020). Extreme environments: microbiology leading to specialized metabolites. *J. Appl. Microbiol.* 128, 630–657.

Schuurmans, D. M., Olson, B. H., & Clemente, C. L. S. (1956). Production and isolation of thermoviridin, an antibiotic produced by Thermoactinomyces viridis n. sp. *Applied microbiology*, *4*(2), 61-66.

Selim, S., Negm, A., & Abouziena, H. (2021). Actinomycetes in agro-ecosystems: A review on their ecology and potential use for sustainable agriculture. Agriculture, 11(8), 718.

Shin, SK., Cho, YJ., & Yi, H. (2017). Synonymy of *Micropolyspora internatus* and *Saccharomonospora viridis* and Emended Description of *Saccharomonospora viridis*. *J. Microbiol. Biotechnol.*;27:149-154.

Shivlata, L., & Satyanarayana, T. (2015). Thermophilic and alkaliphilic *Actinobacteria*: biology and potential applications. *Frontiers in Microbiology*. 6: 1014.

Siliakus, M. F., van der Oost, J., & Kengen, S. W. M. (2017). Adaptations of archaeal and bacterial membranes to variations in temperature, pH and pressure. *Extremophiles : life under extreme conditions*, *21*(4), 651–670.

Smith, D.D.J., & Norton, S.J. (1980). - S- Adenosylmethionine, cyclopropane fatty acid synthase, and the production of lactobacillic acid in *Lactobacillus plantarum*. *Arch. Biochem. Biophys*., 205, 564-570.

Stackebrandt, E., & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.*, 44 (4): 846-849.

Stackebrandt, E., & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* 33, 152–155.

Stackebrandt, E., & Schumann, P. (2006). "Introduction to the taxonomy of *Actinobacteria*," in *The Prokaryotes*, eds M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and F. Stackebrandt (New York, NY: Springer), 297–321.

Subramani, R., & Sipkema, D. (2019). Marine Rare Actinomycetes: A Promising Source of Structurally Diverse and Unique Novel Natural Products. Marine Drugs. 17(5): 249.

Syed, D. G., Tang, S. K., Cai, M., Zhi, X. Y., Agasar, D., Lee, J. C., Kim, C. J., Jiang, C. L., Xu, L. H., & Li, W. J. (2008). *Saccharomonospora saliphila* sp. nov., a halophilic actinomycete from an Indian soil. *IJSEM*, *58*(Pt 3), 570–573.

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101:11030-11035.

Tamura K., Stecher G., & Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*.

Tamura, A., & Takeda, I. (1975). Antibiotic AB-65, a new antibiotic from *Saccharomonospora viridis*. *The Journal of antibiotics*, *28*(5), 395–397.

Tang, S. K., Wang, Y., Klenk, H.P., Shi, R., Lou, K., Zhang, Y.J., Chen, C., Ruan, J.S., & Li, W.J. (2011). *Actinopolyspora alba* sp. nov. and *Actinopolyspora*

erythraea sp. nov., isolated from a salt field, and reclassification of *Actinopolyspora iraqiensis* (Ruan *et al.* 1994) as a heterotypic synonym of *Saccharomonospora halophila*. *IJSEM*. 61, 1693–1698.

Tindall, B. J., Rosselló-Móra, R., Busse, H.-J., Ludwig, W., & Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *Int. J. Syst. Evol. Microbiol., 60*(1), 249-266.

Tiwari, K., & Gupta, RK. (2013). Diversity and isolation of rare actinomycetes: an overview. Crit Rev Biotechnol., 39:256–294.

Tschowri, N. (2016). Cyclic Dinucleotide-Controlled Regulatory Pathways in *Streptomyces* Species. *Journal of Bacteriology*, *198*(1), 47-54.

Tseng, M., Chiang, W.P., Liao, H.C., Hsieh, S.Y., & Yuan, G.F. (2018). *Saccharomonospora piscinae* sp. nov., a novel actinobacterium from fishpond sediment in Taiwan. *Int. J. Syst. Evol. Microbiol.* 68, 1418–1422.

Vane-Wright, R. I. (2013). Taxonomy, Methods of. Encyclopedia of Biodiversity: 7, 97–111.

Vandamme, P., Pot, B., Gillis, M., de Vos, P., Kersters, K., & Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiological Reviews*, *60*(2), 407–438.

Veyisoglu, A., Sazak, A., Cetin, D., Guven, K., & Sahin, N. (2013). *Saccharomonospora amisosensis* sp. nov., isolated from deep marine sediment. *Int. J. Syst. Evol. Microbiol.*, *63*(Pt 10), 3782–3786.

Vishakha R., Tanmaya N., Lopamudra R., Khushbu K.i, & Mrutyunjay S. (2019). A Polyphasic Taxonomic Approach for Designation and Description of Novel Microbial Species. Microbial Diversity in the Genomic Era. Pages: 137-152

Wang, Y. U. E., Zhang, Z., & Ruan, J. (1996). A proposal to transfer *Microbispora bispora* (Lechevalier 1965) to a new genus, *Thermobispora* gen. nov., as *Thermobispora bispora* comb. nov. *IJSEM*, *46*(4), 933-938.

Wang, Y., & Jiang, Y. (2016). Chemotaxonomy of *actinobacteria*. In*Tech*.

Wattanasuepsin, W., Intra, B., Také, A., Inahashi, Y., Euanorasetr, J., Ōmura, S., Matsumoto, A., & Panbangred, W. (2017). *Saccharomonospora colocasiae* sp. nov., an actinomycete isolated from the rhizosphere of *Colocasia esculenta*. *IJSEM*, *67*(11), 4572–4577.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A., & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *Microbiology*, *129*(6), 1743-1813.

Williams, S.T., Goodfellow, M. & Alderson, G. (1989). Genus *Streptomyces* Waksman and Henrici 1943, 339AL. In: Williams S.T., Sharpe, M.E. and Holt, J.G., Eds., Bergey's Manual of Systematic Bacteriology, Vol. 4, Williams and Wilkins, Baltimore, 2452-2492.

Wilson, K. H. (1995). Molecular biology as a tool for taxonomy. *Clinical infectious diseases*, 20(Supplement_2), S117-S121.

Worrall, J. A. R., & Vijgenboom, E. (2010). Copper mining in *Streptomyces*: enzymes, natural products and development. *Natural Product Reports, 27*(5), 742-756.

Xu, LH., Li, WJ., Liu, ZH., & Jiang, CL. (2007). Actinomycete Systematics-Principle, Methods and Practice. 1st ed. Science Press: Beijing.

Yamaguchi, T. (1965). Comparison of the cell-wall composition of morphologically distinct actinomycetes. *Journal of Bacteriology*, *89*(2), 444-453.

Zhang, D. F., Chen, W., He, J., Zhang, X. M., Xiong, Z. J., Sahu, M. K., Sivakumar, K., & Li, W. J. (2013). *Saccharomonospora oceani* sp. nov. isolated from marine sediments in Little Andaman, India. *Antonie van Leeuwenhoek*, *103*(6), 1377–1384.

Zitouni, A., Boudjella, H., Lamari, L., Badji, B., Mathieu, F., Lebrihi, A., & Sabaou, N. (2005). *Nocardiopsis* and *Saccharothrix* genera in Saharan soils in Algeria: isolation, biological activities and partial characterization of antibiotics. *Research in microbiology*, *156*(10), 984-993.


Annexes

Annexe I

Fatty acid content analysis of *Saccharomonosporae*

Species	$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	$C_{14:1}$ *w5c*	$C_{15:0}$ 2 OH
S. amisosensis (Veyisoglu *et al.*, 2013)	-	+	+	+	+	+	-	-
S. azurea (Embley *et al.*, 1988)	-	+	+	+	+	-	-	+
S. colocasiae (Wattanasuepsin *et al.*, 2017)	-	-	+	+	-	-	-	+
S. cyanea (Runmao *et al.*, 1988)(Meier-Kolthoff *et al.*, 2013)	-	-	+	+	+	-	-	-
S. glauca (Greiner-Mai *et al.*, 1988)	-	-	+	+	-	+	-	+
S. halophila (Al-Zarban *et al.*, 2002)(Tand *et al.*, 2011)	+	+	+	+	+	+	-	-
S. iraqiensis subsp. *iraqiensis* (Ruan *et al.*, 1994)	-	+	-	-	-	-	-	-
S. iraqiensis subsp. *paurometabolica* (Li *et al.*, 2003)	-	-	-	+	-	+	-	-
S. marina (Lieu *et al.*, 2010)(Veyisoglu *et al.*, 2013)(Zhang *et al.*, 2013)	-	+	+	+	+	+	+	-
S. oceani (Zhang *et al.*, 2013)	-	+	-	-	-	-	+	-
S. piscinae (Tseng *et al.*, 2018)	-	-	-	-	-	-	-	-
S. saliphila (Syed *et al.*, 2008)	-	-	-	-	-	-	-	-
S. viridis (Embley *et al.*, 1988)(*Pati*, 2009)(Wattanasuepsin *et al.*, 2017)	-	-	+	+	-	-	-	+
S. xiaoerkulensis (Li *et al.*, 2016)	-	-	-	+	+	-	-	-
S. xinjiangensis (Jin *et al.*, 1998)(Lieu *et al.*, 2010)	-	-	+	+	+	-	-	-
Frequency	**1/15**	**6/15**	**9/15**	**11/15**	**7/15**	**5/15**	**2/15**	**4/15**
	6.66%	**40%**	**60%**	**73.33%**	**46.66%**	**33.33%**	**13.33%**	**26.66%**

Fatty acid content analysis of *Saccharomonosporae* –continued-

Species	$C_{15:1}$ *w6c*	$C_{16:0\ 2\ OH}$	$C_{16:1}$	$C_{16:1}$ *cis* 9	$C_{16:1}$ *w6c*	$C_{16:1}$ *w7c*	$C_{16:1}$ *w9c*	$C_{17:1}$	$C_{17:0}$ *w8c*
S. amisosensis (Veyisoglu *et al.*, 2013)	-	-	-	+	-	-	-	-	-
S. azurea (Embley *et al.*, 1988)	-	-	-	+	-	-	+	-	-
S. colocasiae (Wattanasuepsin *et al.*, 2017)	-	-	-	-	-	-	-	-	-
S. cyanea (Runmao *et al.*, 1988)(Meier-Kolthoff *et al.*, 2013)	-	-	-	+	-	-	-	-	-
S. glauca (Greiner-Mai *et al.*, 1988)	-	-	-	-	-	-	+	-	-
S. halophila (Al-Zarban *et al.*, 2002)(Tand *et al.*, 2011)	-	+	+	-	-	-	-	+	-
S. iraqiensis subsp. *iraqiensis* (Ruan *et al.*, 1994)	-	-	-	-	-	-	-	-	-
S. iraqiensis subsp. *paurometabolica* (Li *et al.*, 2003)	-	-	+	-	-	-	-	-	-
S. marina (Veyisoglu *et al.*, 2013)(Zhang *et al.*, 2013)	+	-	-	+	+	-	-	-	-
S. oceani (Zhang *et al.*, 2013)	+	-	-	-	**SM**	**SM**	-	-	-
S. piscinae (Tseng *et al.*, 2018)	+	-	-	-	**SM**	**SM**	-	-	+
S. saliphila (Syed *et al.*, 2008)	-	-	-	-	-	-	-	-	-
S. viridis (Embley *et al.*, 1988)(Wattanasuepsin *et al.*, 2017)	-	-	-	-	-	-	-	-	-
S. xiaoerkulensis (Li *et al.*, 2016)	-	-	-	-	-	-	-	-	-
S. xinjiangensis (Jin *et al.*, 1998)(Lieu *et al.*, 2010)	-	-	-	-	-	-	-	-	-
Frequency	**3/15**	**1/15**	**2/15**	**4/15**	**3/15**	**2/15**	**2/15**	**1/15**	**1/15**
	20%	**6.66 %**	**13.33 %**	**26.66 %**	**20%**	**13.33 %**	**13.33 %**	**6.66 %**	**6.66 %**

SM,summed features.

Fatty acid content analysis of *Saccharomonosporae* –continued-

Species	$C_{17:1}$ *w6c*	$C_{17:1}$ *cis* 9	$C_{17:1}$ *w*8c	$C_{17:1}$ *w9c*	$C_{18:1}$	$C_{18:1}$ *cis* 9	$C_{18:1}$ *w9*c	iso-C14:0	iso-C15:0
S. amisosensis (Veyisoglu *et al.*, 2013)	-	+	-	-	-	+	-	+	+
S. azurea (Embley *et al.*, 1988)	-	-	-	+	-	-	-	-	+
S. colocasiae (Wattanasuepsin *et al.*, 2017)	-	-	-	+	-	-	-	+	+
S. cyanea (Runmao *et al.*, 1988)(Meier-Kolthoff *et al.*, 2013)	-	+	-	+	-	-	-	-	+
S. glauca (Greiner-Mai *et al.*, 1988)	-	-	-	+	-	-	-	+	+
S. halophila (Al-Zarban *et al.*, 2002) (Tand *et al.*, 2011)	+	-	+	-	+	+	-	+	+
S. iraqiensis subsp. *iraqiensis* (Ruan *et al.*, 1994)	-	-	-	-	-	-	-	+	-
S. iraqiensis subsp. *paurometabolica* (Li *et al.*, 2003)	-	-	-	-	-	-	-	-	+
S. marina (Lieu *et al.*, 2010)(Veyisoglu *et al.*, 2013)(Zhang *et al.*, 2013)	+	+	+	-	-	-	+	+	+
S. oceani (Zhang *et al.*, 2013)	+	-	+	-	-	-	-	+	+
S. piscinae (Tseng *et al.*, 2018)	+	-	+	-	-	-	-	+	+
S. saliphila (Syed *et al.*, 2008)	+	-	-	-	-	-	-	-	+
S. viridis (Embley *et al.*, 1988)(Wattanasuepsin *et al.*, 2017)	-	-	-	+	-	-	-	-	+
S. xiaoerkulensis (Li *et al.*, 2016)	+	-	+	-	-	+	+	-	+
S. xinjiangensis (Jin *et al.*, 1998)(Lieu *et al.*, 2010)	+	-	+	-	-	-	-	-	-
Frequency	**7/15**	**3/15**	**6/15**	**5/15**	**1/15**	**2/15**	**2/15**	**8/15**	**13/15**
	46.66 %	**20%**	**40%**	**33.33 %**	**6.66%**	**13.33 %**	**13.33 %**	**53.33 %**	**86.66 %**

Fatty acid content analysis of *Saccharomonosporae* –continued-

Species	anteiso-$C_{15:0}$	anteiso-$C_{15:0\ 2OH}$	iso-$C_{16:0}$	anteiso-$C_{16:0}$	iso-$C_{16:0\ 2OH}$	iso-$C_{16:1}$	iso-$C_{16:1\ H}$	anteiso-$C_{16:0}$	iso-$C_{17:0}$
S. amisosensis (Veyisoglu *et al.*, 2013)	-	-	+	-	+	-	+	-	+
S. azurea (Embley *et al.*, 1988)	+	-	+	+	+	-	+	-	+
S. colocasiae (Wattanasuepsin *et al.*, 2017)	+	-	+	-	+	-	+	-	-
S. cyanea (Runmao *et al.*, 1988)(Meier-Kolthoff *et al.*, 2013)	+	-	+	-	+	-	-	-	+
S. glauca (Greiner-Mai *et al.*, 1988)	+	+	+	-	+	-	+	-	+
S. halophila (Al-Zarban *et al.*, 2002)(Tand *et al.*, 2011)	+	+	+	-	-	-	-	+	+
S. iraqiensis subsp. *iraqiensis* (Ruan *et al.*, 1994)	+	-	-	-	-	-	-	+	+
S. iraqiensis subsp. *paurometabolica* (Li *et al.*, 2003)	-	+	+	-	-	+	-	-	+
S. marina (Lieu et *al.*, 2010)(Veyisoglu *et al.*, 2013)(Zhang *et al.*, 2013)	-	+	+	-	+	-	+	-	+
S. oceani (Zhang *et al.*, 2013)	-	+	+	-	-	-	+	-	+
S. piscinae (Tseng *et al.*, 2018)	-	-	+	-	-	-	+	+	+
S. saliphila (Syed *et al.*, 2008)	-	-	+	-	-	-	-	-	+
S. viridis (Embley *et al.*, 1988)(Pati, 2009)(Wattanasuepsin *et al.*, 2017)	-	+	+	-	+	-	+	-	+
S. xiaoerkulensis (Li *et al.*, 2016)	-	-	+	-	-	-	+	-	+
S. xinjiangensis (Jin *et al.*, 1998)(Lieu *et al.*, 2010)	-	-	+	-	-	-	-	-	-
Frequency	**6/15**	**6/15**	**14/15**	**1/15**	**7/15**	**1/15**	**9/15**	**3/15**	**13/15**
	40%	40%	93.33%	6.66%	46.66%	6.66%	60%	20%	86.66%

Fatty acid content analysis of *Saccharomonosporae* –continued-

Species	iso-$C_{17:0\ 2OH}$	iso-$C_{17:1}$	iso-$C_{17:1}$ *w9*c	anteiso-$C_{17:0}$	anteiso-$C_{17:0\ 2\text{-}OH}$	anteiso-$C_{17:1\ c}$	iso-$C_{18:0}$	10-Methyl $C_{16:0}$	10-Methyl $C_{17:0}$
S. amisosensis (Veyisoglu *et al.*, 2013)	-	-	-	+	-	-	+	+	-
S. azurea (Embley *et al.*, 1988)	-	-	-	-	-	-	-	-	-
S. colocasiae (Wattanasuepsin *et al.*, 2017)	-	-	-	+	-	-	-	-	-
S. cyanea (Runmao *et al.*, 1988)(Meier-Kolthoff *et al.*, 2013)	-	-	-	+	-	-	-	-	-
S. glauca (Greiner-Mai *et al.*, 1988)	-	-	-	+	-	-	+	-	-
S. halophila (Al-Zarban *et al.*, 2002)(Tand *et al.*, 2011)	+	-	-	+	+	+	+	+	-
S. iraqiensis subsp. *iraqiensis* (Ruan *et al.*, 1994)	-	-	-	+	-	-	+	-	-
S. iraqiensis subsp. *paurometabolica* (Li *et al.*, 2003)	-	+	-	+	-	-	-	-	-
S. marina (Lieu *et al.*, 2010)(Veyisoglu *et al.*, 2013)	-	-	-	+	-	-	-	**SM**	+
S. oceani (Zhang *et al.*, 2013)	-	-	**SM**	+	-	-	+	**SM**	+
S. piscinae (Tseng *et al.*, 2018)	-	-	-	+	-	-	-	-	-
S. saliphila (Syed *et al.*, 2008)	-	-	-	-	-	-	+	+	-
S. viridis (Embley *et al.*, 1988)(Wattanasuepsin *et al.*, 2017)	-	-	-	+	-	-	+	-	-
S. xiaoerkulensis (Li *et al.*, 2016)	-	-	-	+	-	-	+	-	-
S. xinjiangensis (Jin *et al.*, 1998)(Lieu *et al.*, 2010)	-	-	-	-	-	-	-	-	-
Frequency	**1/15**	**1/15**	**1/15**	**12/15**	**1/15**	**1/15**	**7/15**	**4/15**	**2/15**
	6.66%	6.66%	6.66%	80%	6.66%	6.66%	46.6%	26.6%	13.3%

SM,summed features.

Annexe II

16S rRNA gene sequences of the studied species, and *Actinopolyspora algeriensis*.

Saccharomonospora amisosensis DS3030

```
   1 gctcaggacg aacgctggcg gcgtgcttaa cacatgcaag tcggacgctg aagctcagct
  61 tgctgggtgg atgagtggcg aacgggtgag taacacgtgg gtaatctgcc ctgtacttcg
 121 ggataagcct tggaaacggg gtctaatacc ggataggaca catcgtcgca tggtggtgtg
 181 tggaaagcct ttgggtggta tgggatgagc ccgcggccta tcagcttgtt ggtggggtga
 241 tggcctacca aggcggtgac gggtagccgg cctgagaggg tgaccggcca cactgggact
 301 gagacacggc ccagactcct acgggaggca gcagtgggga atattgcaca atgggcgcaa
 361 gcctgatgca gcgacgccgc gtgagggatg acggccttcg ggttgtaaac ctctttcgcc
 421 caggacgaag ggtttcggct tgacggtact gggagaagaa gcaccggcta actacgtgcc
 481 agcagccgcg gtaatacgta gggtgcgagc gttgtccgga attattgggc gtaaagagct
 541 cgtaggcggt gtgtcacgtc tgccgtgaaa acctacggct taaccgtggg cgtgcggtgg
 601 atacgggcat cacttgagtt cggtagggga gactggaatt cctggtgtag cggtggaatg
 661 cgcagatatc aggaggaaca ccggtggcga aggcgggtct ctgggccgat actgacgctg
 721 aggagcgaaa gcgtggggag cgaacaggat tagataccct ggtagtccac gctgtaaacg
 781 ttgggcgcta ggtgtggggt gctgttcacg tgtcccgtgc cgtagctaac gcattaagcg
 841 ccccgcctgg ggagtacggc cgcaaggcta aaactcaaag gaattgacgg gggcccgcac
 901 aagcggcgga gcatgtggat taattcgatg caacgcgaag aaccttacct gggcttgaca
 961 tgcatcagac gcatccagag atgggtgttc ccttgtggtt ggtgtacagg tggtgcatgg
1021 ctgtcgtcag ctcgtgtcgt gagatgttgg gttaagtccc gcaacgagcg caacccttgt
1081 cctatgttgc cagcgggtta tgccggggac tcgtgggaga ctgccggggt caactcggag
1141 gaaggtgggg atgacgtcaa gtcatcatgc cccttatgtc cagggcttca cacatgctac
1201 aatggctggt acagagggtg gcgataccgt gaggtggagc gaatccctta aagccggtct
1261 cagttcggat cgtagtctgc aactcgactg cgtgaagtcg gagtcgctag taatcgcaga
1321 tcagcagtgc tgcggtgaat acgttcccgg gccttgtaca caccgcccgt cacgtcacga
1381 aagtcggtaa cacccgaagc ccatggccta acccacgttg gtgggggga gtggtcgaag
1441 gtgggactgg cgattgggac gaagtcgtaa caaggta
```

Saccharomonospora azurea NA128

```
   1 gctcaggacg aacgctggcg gcgtgcttaa cacatgcaag tcgaacgctg aagcccagct
  61 tgctgggtgg atgagtggcg aacgggtgag taacacgtgg gtaatctgcc ctgtactctg
 121 ggataagcct gggaaactgg gtctaatacc ggataggaca cactgccgca tggtggtgtg
 181 tggaaagctc cggcggtaca ggttgagccc gcggcctatc agcttgttgg tggggtgatg
 241 gcctaccaag gcgacgacgg gtagccggcc tgagagggtg accggccaca ctgggactga
 301 gacacggccc agactcctac gggaggcagc agtggggaat attgcacaat gggcgcaagc
 361 ctgatgcagc gacgccgcgt gggggatgac ggccttcggg ttgtaaaccc ctttcgccag
 421 ggacgaagcg taagtgacgg tacctggaga agaagcaccg gccaactacg tgccagcagc
 481 cgcggtaata cgtagggtgc aagcgttgtc cggaattatt gggcgtaaag agctcgtagg
 541 cggtgtgtca cgtctgccgt gaaaacctgc ggcttaaccg tgggcgtgcg gtggatacgg
 601 gcatcacttg agttcggtag gggagactgg aattcctggt gtagcggtgg aatgcgcaga
```

```
 661 tatcaggagg aacaccggtg gcgaaggcgg gtctctgggc cgatactgac gctgaggagc
 721 gaaagcgtgg ggagcgaaca ggattagata ccctggtagt ccacgccgta aacgttgggc
 781 gctaggtgtg gggcgctgtt cacgtgtccc gtgccgtagc taacgcatta agcgccccgc
 841 ctggggagta cggccgcaag gctaaaactc aaaggaattg acgggggccc gcacaagcgg
 901 cggagcatgt ggattaattc gatgcaacgc gaagaacctt acctgggctt gacatgcacc
 961 ggatcgcctc agagatgggg tttcccttgt ggtcggtgca caggtggtgc atggctgtcg
1021 tcagctcgtg tcgtgagatg ttgggttaag tcccgcaacg agcgcaaccc ttgtcccatg
1081 ttgccagcgg gtaatgccgg ggactcgtgg gagactgccg gggtcaactc ggaggaaggt
1141 ggggatgacg tcaagtcatc atgcccctta tgtccagggc ttcacacatg ctacaatggc
1201 tggtacagag ggttgcgata ccgtgaggtg gagcgaatcc cttaaagcca gtctcagttc
1261 ggatcgcagt ctgcaactcg actgcgtgaa gtcggagtcg ctagtaatcg cagatcagca
1321 ttgctgcggt gaatacgttc ccgggccttg tacacaccgc ccgtcacgtc atgaaagtcg
1381 gtaacacccg aagcccatgg cccaacccgc ttgcgggggg gagtggtcga aggtgggact
1441 ggcgattggg acgaagtcgt aacaaggtag ccgtaccgga aggtgcggct g
```

Saccharomonospora colocasiae S265

```
   1 tgcagtcgac gctgaagccc agcttgctgg gtggatgagt ggcgaacggg ngagtaacac
  61 gtgggtaatc tgccctgtac tctgggataa gcctgggaaa ctgggtctaa taccggatag
 121 gacattccac cgcatggtgg ggtgtggaaa gctccggcgg tacaggttga gcccgcggcc
 181 tatcagcttg ttggtggggt gatggcctac caaggcgacg acgggtagcc ggcctgagag
 241 ggtgaccggc cacactggga ctgagacacg gcccagactc ctacgggagg cagcagtggg
 301 gaatattgca caatgggcgc aagcctgatg cagcgacgcc gcgtggggga tgacggcctt
 361 cgggttgtaa acccctttcg ccagggacga agcgagagtg acggtacctg gagaagaagc
 421 accggccaac tacgtgccag cagccgcggt aatacgtagg gtgcaagcgt tgtccggaat
 481 tattgggcgt aaagagctcg taggcggtgt gtcacgtctg ccgtgaaaac ctgcggctta
 541 accgtgggcg tgcggtggat acgggcatca cttgagttcg gtaggggaga ctggaattcc
 601 tggtgtagcg gtggaatgcg cagatatcag gaggaacacc ggtggcgaag gcgggtctct
 661 gggccgatac tgacgctgag gagcgaaagc gtggggagcg aacaggatta gataccctgg
 721 tagtccacgc cgtaaacgtt gggcgctagg tgtggggcgc tgttcacgtg tcccgtgccg
 781 tagctaacgc attaagcgcc ccgcctgggg agtacggccg caaggctaaa actcaaagga
 841 attgacgggg gcccgcacaa gcggcggagc atgtggatta attcgatgca acgcgaagaa
 901 ccttacctgg gcttgacatg cactggaccg gcgtagagat acgtcttccc ttgtggctgg
 961 tgcacaggtg gtgcatggct gtcgtcagct cgtgtcgtga gatgttgggt taagtcccgc
1021 aacgagcgca acccttgtcc catgttgcca gcgggtaatg ccggggactc gtgggagact
1081 gccggggtca actcggagga aggtggggat gacgtcaagt catcatgccc cttatgtcca
1141 gggcttcaca catgctacaa tgggctggta cagagggttg cgagaccgtg aggtggagcg
1201 aatcccttaa agccagtctc agttcggatc gcagtctgca actcgactgc gtgaagtcgg
1261 agtcgctagt aatcgcagat cagcattgct gcggtgaata cgttcccggg ccttgtacac
1321 accgcccgtc acgtcatgaa agtcggtaac acccgaagcc catggcccaa ccccttgt
```

Saccharomonospora cyanea NA134

```
   1 gctcaggacg aacgctggcg gcgtgcttaa cacatgcaag tcgaacgctg aagcccagct
  61 tgctgggtgg atgagtggcg aacgggtgag taacacgtgg gtaatctgcc ctgtactctg
 121 ggataagccc gggaaactgg gtctaatacc ggataggacg cctcaccgca tggtggggtg
 181 tggaaagttc cggcggtaca ggttgagccc gcggcctatc agcttgttgg tggggtgatg
 241 gcctaccaag gcgacgacgg gtagccggcc tgagagggtg accggccaca ctgggactga
 301 gacacggccc agactcctac gggaggcagc agtggggaat attgcacaat gggcgcaagc
 361 ctgatgcagc gacgccgcgt gggggatgac ggccttcggg ttgtaaaccc ctttcgcccg
 421 ggacgaagcg caagtgacgg taccgggaga agaagcaccg gccaactacg tgccagcagc
 481 cgcggtaata cgtagggtgc aagcgttgtc cggaattatt gggcgtaaag agctcgtagg
 541 cggtgtgtca cgtctgccgt gaaaacctgc ggcttaaccg tgggcgtgcg gtggatacgg
 601 gcatcacttg agttcggtag gggagactgg aattcctggt gtagcggtgg aatgcgcaga
 661 tatcaggagg aacaccggtg gcgaaggcgg gtctctgggc cgaaactgac gctgaggagc
 721 gaaagcgtgg ggagcgaaca ggattagata ccctggtagt ccacgccgta aacgttgggc
 781 gctaggtgtg gggtgctgtt cacgcgtccc gtgccgtagc taacgcatta agcgccccgc
 841 ctggggagta cggccgcaag gctaaaactc aaaggaattg acgggggccc gcacaagcgg
 901 cggagcatgt ggattaattc gatgcaacgc gaagaacctt acctgggctt gacatgcacc
 961 ggatcgcctc agagatgggg tttcccttgt ggctggtgca caggtggtgc atggctgtcg
1021 tcagctcgtg tcgtgagatg ttgggttaag tcccgcaacg agcgcaaccc ttgtcccatg
1081 ttgccagcgg gtaatgccgg ggactcgtgg gagactgccg gggtcaactc ggaggaaggt
1141 ggggatgacg tcaagtcatc atgcccctta tgtccagggc ttcacacatg ctacaatggc
1201 cggtacagtg ggtggcgata ccgtgaggtg gagcgaatcc ctcaaagccg gtctcagttc
1261 ggatcgcagt ctgcaactcg actgcgtgaa gtcggagtcg ctagtaatcg cagatcagca
1321 ttgctgcggt gaatacgttc ccgggccttg tacacaccgc ccgtcacgtc atgaaagtcg
1381 gtaacacccg aagcccatgg cccaaccctt cggggaggga gtggtcgaag gtgggactgg
1441 cgattgggac gaagtcgtaa caaggtagcc gtaccggaag gtgcggctg
```

Saccharomonospora glauca K62

```
   1 gctcaggacg aacgctggcg gcgtgcttaa cacatgcaag tcgaacgctg aagcccagct
  61 tgctgggtgg atgagtggcg aacgggtgag taacacgtgg gtaatctgcc ccgtactccg
 121 ggataagccc gggaaactgg gtctaatacc ggataggaca cgctatcgca tggtggtgtg
 181 tggaaagctc cggcggtacg ggatgagccc gcggcctatc agcttgttgg tggggtgatg
 241 gcctaccaag gcgacgacgg gtagccggcc tgagagggtg accggccaca ctgggactga
 301 gacacggccc agactcctac gggaggcagc agtggggaat attgcacaat gggcgcaagc
 361 ctgatgcagc gacgccgcgt gggggatgac ggccttcggg ttgtaaaccc ctttcgcccg
 421 ggacgaagcg taagtgacgg taccgggaga agaagcaccg gccaactacg tgccagcagc
 481 cgcggtaata cgtagggtgc aagcgttgtc cggaattatt gggcgtaaag agctcgtagg
 541 cggtgtgtca cgtctgccgt gaaaacctgc ggcttaaccg tgggcgtgcg gtggatacgg
 601 gcatcacttg agttcggtag gggagactgg aattcctggt gtagcggtgg aatgcgcaga
 661 tatcaggagg aacaccggtg gcgaaggcgg gtctctgggc cgatactgac gctgaggagc
 721 gaaagcgtgg ggagcgaaca ggattagata ccctggtagt ccacgccgta aacgttgggc
 781 gctaggtgtg gggtgctgtt cacgtgtccc gtgccgtagc taacgcatta agcgccccgc
```

```
 841 ctggggagta cggccgcaag gctaaaactc aaaggaattg acgggggccc gcacaagcgg
 901 cggagcatgt ggattaattc gatgcaacgc gaagaacctt acctgggctt gacatgcacc
 961 ggatcggcgt agagatacgt cttcccttgt ggctggtgca caggtggtgc atggctgtcg
1021 tcagctcgtg tcgtgagatg ttgggttaag tcccgcaacg agcgcaaccc ttgtcccatg
1081 ttgccagcgg gttatgccgg ggactcgtgg gagactgccg gggtcaactc ggaggaaggt
1141 ggggatgacg tcaagtcatc atgcccctta tgtccagggc ttcacacatg ctacaatggc
1201 cggtacaatg ggctgcgata ccgtgaggtg gagcgaatcc ctgaaagccg gtctcagttc
1261 ggatcgtagt ctgcaactcg actgcgtgaa gtcggagtcg ctagtaatcg cagatcagca
1321 ttgctgcggt gaatacgttc ccgggccttg tacacaccgc ccgtcacgtc atgaaagtcg
1381 gtaacacccg aagcccatgg cccaacccct tgtgggaggg agtggtcgaa ggtgggactg
1441 gcgattggga cgaagtcgta acaaggtagc cgtaccggaa ggtgcggctg
```

Saccharomonospora halophila DSM 44411

```
   1 gtttgatcct ggctcaggac gaacgctggc ggcgtgctta acacatgcaa gtcgaacgct
  61 gaagccgctt tcgggtggtg gatgagtggc gaacgggtga gtaacacgtg ggcaatctgc
 121 cctgtactct gggataagcc ttggaaacgg ggtctaatac cggatgggac actgcttcgc
 181 atggggtggt gtggaaagct tcggcggtac gggatgagcc cgcggcctat cagcttgttg
 241 gtggggtgat ggcctaccaa ggcgacgacg ggtagccggc ctgagagggt gaccggccac
 301 actgggactg agacacggcc cagactccta cgggaggcag cagtggggaa tattgcacaa
 361 tgggcggaag cctgatgcag cgacgccgcg tgagggatga cggccttcgg gttgtaaacc
 421 tctttcgccc gggacgaagc gtcacagtga cggtaccggg agaagaagca ccggctaact
 481 acgtgccagc agccgcggta atacgtaggg tgcgagcgtt gtccggaatt attgggcgta
 541 aagagctcgt aggcggtgtg tcacgtctgc cgtgaaaacc tgcggcttaa ccgtgggcgt
 601 gcggtggata cgggcatcac ttgagttcgg caggggagac tggaattcct ggtgtagcgg
 661 tggaatgcgc agatatcagg aggaacaccg gtggcgaagg cgggtctctg ggccgatact
 721 gacgctgagg agcgaaagcg tggggagcga acaggattag ataccctggt agtccacgcc
 781 gtaaacgttg ggcgctaggt gtgggacgct gttcacgtgt cccgtgccgt agctaacgca
 841 ttaagcgccc cgcctgggga gtacggccgc aaggctaaaa ctcaaaggaa ttgacggggg
 901 cccgcacaag cggcggagca tgtggattaa ttcgatgcaa cgcgaagaac cttacctggg
 961 cttgacatgc accggatcgc ctcagagatg gggtttccct tgtggctggt gcacaggtgg
1021 tgcatggctg tcgtcagctc gtgtcgtgag atgttgggtt aagtcccgca acgagcgcaa
1081 cccttgtccc atgttgccag cgggttatgc cggggactcg tgggagactg ccggggtcaa
1141 ctcggaggaa ggtggggatg acgtcaagtc atcatgcccc ttatgtccag ggcttcacac
1201 atgctacaat ggccggtaca gagggtggcg agaccgtgag gtggagcgaa tcccttaaag
1261 ccggtctcag ttcggatcgt agtctgcaac tcgactgcgt gaagtcggag tcgctagtaa
1321 tcgcagatca gcaacgctgc ggtgaatacg ttcccgggcc ttgtacacac cgcccgtcac
1381 gtcatgaaag tcggtaacac ccgaagctca cggcccaacc gttcgcgggg ggagtggtcg
1441 aaggtgggac tggcgattgg gacgaagtcg taacaaggta gccgtaccgg aaggtgcggc
1501 tggatc
```

Saccharomonospora iraqiensis subsp. *iraqiensis* IQ-H1= DSM 44640

```
   1 tacacatgca agtcgaacgc tgaagccacc ttcgggtggt ggatgagtgg cgaacgggtg
  61 agtaacacgt gggcaatctg ccctgtactc tgggataagc cctggaaacg gggtctaata
 121 ccggatagga catcgcctcg catggggtgg tgtggaaagc ttcggcggta cgggatgagc
 181 ccgcggccta tcagcttgtt ggtggggtga tggcctacca aggcgacgac gggtagccgg
 241 cctgagaggg tgaccggcca cactgggact gagacacggc ccagactcct acgggaggca
 301 gcagtgggga atattgcaca atgggcggaa gcctgatgca gcgacgccgc gtgagggatg
 361 acggccttcg ggttgtaaac ctctttcgcc cgggacgaag cgtaaaagtg acggtaccgg
 421 gagaagaagc accggctaac tacgtgccag cagccgcggt aatacgtagg gtgcgagcgt
 481 tgtccggaat tattgggcgt aaagagctcg taggcggtgt gtcacgtctg ccgtgaaaac
 541 ctgcggctta accgtgggcg tgcggtggat acgggcatca cttgagttcg gcaggggaga
 601 ctggaattcc tggtgtagcg gtggaatgcg cagatatcag gaggaacacc ggtagcgaag
 661 gcgggtctct gggccgatac tgacgctgag gagcgaaagc gtggggagca aacaggatta
 721 gataccctgg tagtccacgc cgtaaacgtt gggcgctagg tgtgggacgc tgttcacgtg
 781 tcccgtgccg tagctaacgc attaagcgcc ccgcctgggg agtacggccg caaggctaaa
 841 actcaaagga attgacgggg gcccgcacaa gcggcggagc atgtggatta attcgatgca
 901 acgcgaagaa ccttacctgg gcttgacatg caccggatcg cctcagagat ggggtttccc
 961 ttgtggctgg tgcacaggtg gtgcatggct gtcgtcagct cgtgtcgtga gatgttgggt
1021 taagtcccgc aacgagcgca acccttgtcc catgttgcca gcgggttatg ccggggactc
1081 gtgggagact gccggggtca actcggagga aggtggggat gacgtcaagt catcatgccc
1141 cttatgtcca gggcttcaca catgctacaa tggccggtac agagggtggc gagaccgtga
1201 ggtggagcga atcccttaaa gccggtctca gttcggatcg tagtctgcaa ctcgactgcg
1261 tgaagtcgga gtcgctagta atcgcagatc agcaacgctg cggtgaatac gttcccgggc
1321 cttgtacaca ccgcccgtca cgtcatgaaa gtcggtaaca cccgaagccc acggcccaac
1381 cgttcgcggg gggagtggtc gaaggtggga ctggcgattg ggacgaagtc gtaa
```

Saccharomonospora iraqiensis subsp. *paurometabolica* YIM 90007

```
   1 ttagagtttt gatcctggct caggacgaac gctggcggcg tgcttaacac atgcaagtcg
  61 aacgctgaag ccgcttcggt ggtggatgag tggcgaacgg gtgagtaaca cgtgggcaat
 121 ctgccctgta ctctgggata agccctggaa acggggtcta ataccggata ggacactgtc
 181 tcgcatgggg tggtgtggaa agcttcggcg gtacgggatg agcccgcggc ctatcagctt
 241 gttggtgggg tgatggccta ccaaggcgac gacgggtagc cggcctgaga gggtgaccgg
 301 ccacactggg actgagacac ggcccagact cctacgggag gcagcagtgg ggaatattgc
 361 acaatgggcg gaagcctgat gcagcgacgc cgcgtgaggg atgacggcct tcgggttgta
 421 aacctctttc gcccgggacg aagcgtcaca gtgacggtac cgggagaaga agcaccggct
 481 aactacgtgc cagcagccgc ggtaatacgt agggtgcgag cgttgtccgg aattattggg
 541 cgtaaagagc tcgtaggcgg tgtgtcacgt ctgccgtgaa aacctgcggc ttaaccgtgg
 601 gcgtgcggtg gatacgggca tcacttgagt tcggcagggg agactggaat tcctggtgta
 661 gcggtggaat gcgcagatat caggaggaac accggtggcg aaggcgggtc tctgggccga
 721 tactgacgct gaggagcgaa agcgtgggga gcgaacagga ttagataccc tggtagtcca
 781 cgccgtaaac gttgggcgct aggtgtggga cgctgttcac gtgtcccgtg ccgtagctaa
 841 cgcattaagc gccccgcctg gggagtacgg ccgcaaggct aaaactcaaa ggaattgacg
```

```
 901 ggggcccgca caagcggcgg agcatgtgga ttaattcgat gcaacgcgaa gaaccttacc
 961 tgggcttgac atgcaccgga tcgcctcaga gatggggttt cccttgtggc tggtgcacag
1021 gtggtgcatg gctgtcgtca gctcgtgtcg tgagatgttg ggttaagtcc cgcaacgagc
1081 gcaacccttg tnccatgttg ccagcgggtt atgccgggga ctcttgggag actgncgggg
1141 tcaactcgga ggaaggtggg gatgacntca agtcatcatg ccccttatgt ccagggcttt
1201 acacatgcta caatggccgg tacanagggt ggcgagaccg ngaggtggag cgaatccctt
1261 aaagccggtc tcagttcgga tcgtagnctg naactcnact gtgtgaagtc ggagtcncta
1321 gtaatcgcag atcagcaacg ctgcggtgaa tacgttcccg ggccttgtac acaccgcccg
1381 tcacgtcatg aaagtcggta acacccgaag cccacggccc aaccgttcgc ggggggagtg
1441 gtcaaaggtg ggactggcga ttgggacaaa gtc
```

Saccharomonospora marina XMU15

```
   1 gagtttgatc gtggctcagg acgaacgctg gcggcgtgct taacacatgc aagtcggacg
  61 ctgaagctca gcttgctggg tggatgagtg gcgaacgggt gagtaacacg tgggtaatct
 121 gccctgtact tcgggataag ccttggaaac ggggtctaat accgggtagg acacatcgtc
 181 gcatggtggt gtgtggaaag cctttcgggg tggtatggga tgagcccgcg gcctatcagc
 241 ttgttggtgg ggtgatggcc taccaaggcg gtgacgggta gccggcctga gagggtgacc
 301 ggccacactg ggactgagac acggcccaga ctcctacggg aggcagcagt ggggaatatt
 361 gcacaatggg cgcaagcctg atgcagcgac gccgcgtgag ggatgacggc cttcgggttg
 421 taaacctctt tcgcccagga cgaaagggtt tcgggcttga cggtactgga gaagaagcac
 481 cggctaacta cgtgccagca gccgcggtaa tacgtagggt gcgagcgttg tccggaatta
 541 ttgggcgtaa agagctcgta ggcggtgtgt cacgtctgcc gtgaaaacct acggcttaac
 601 cgtgggcgtg cggtggatac gggcatcact tgagttcggt aggggagact ggaattcctg
 661 gtgtagcggt ggaatgcgca gatatcagga ggaacaccgg tggcgaaggc gggtctctgg
 721 gccgatactg acgctgagga gcgaaagcgt ggggagcgaa caggattaga taccctggta
 781 gtccacgctg taaacgttgg gcgctaggtg tggggtgctg ttcacgtgtc ccgtgccgta
 841 gctaacgcat taagcgcccc gcctggggag tacggccgca aggctaaaac tcaaaggaat
 901 tgacgggggc ccgcacaagc ggcggagcat gtggattaat tcgatgcaac gcgaagaacc
 961 ttacctgggc ttgacatgca tcagacgcat ccagagatgg gtgttccctt gtggttggtg
1021 tactggtggt gcatggctgt cgtcagctcg tgtcgtgaga tgttgggtta agtcccgcaa
1081 cgagcgcaac ccttgtccta tgttgccagc gggtaatgcc ggggactcgt gggagactgc
1141 cggggtcaac tcggaggaag gtggggatga cgtcaagtca tcatgccccc tatgtccagg
1201 gcttcacaca tgctacaatg gctggtacag agggtggcga taccgtgagg tggagcgaat
1261 cccttaaagc cggtctcagt tcggatcgta gtctgcaact cgactgcgtg aagtcggagt
1321 cgctagtagt cgcagatcag cagtgctgcg gtgaatacgt tcccgggcct tgtacacacc
1381 gcccgtcacg tcacgaaagt cggtaacacc cgaagcccat ggcccaaccc ctgttgggga
1441 gggagtggtc gaaggtggga ctggcgattg ggacgaagtc gtaacaaggt agccgtaccg
1501 gaaggtgcgg ctggatcacc tccttaatc
```

Saccharomonospora oceani YIM M11168

```
   1 aacgctggcg gcgtgcttaa cacatgcaag tcgtacgctg aagccgcttc ggtggtggat
  61 gagtggcgaa cgggtgagta acacgtgggt aatctgccct gtactttggg ataagccttg
```

```
 121 gaaacggggt ctaataccgg ataggacatg ctctcgcatg ggggtgtgtg gaaagctccg
 181 gcggtacggg ttgagcccgc ggcctatcag cttgttggtg gggtgatggc ctaccaaggc
 241 ggtgacgggt agccggcctg agagggcgac cggccacact gggactgaga cacggcccag
 301 actcctacgg gaggcagcag tggggaatat tgcacaatgg gcgcaagcct gatgcagcga
 361 cgccgcgtgg gggatgacgg ccttcgggtt gtaaacctct ttcgcccggg acgaagggag
 421 actgacggta ccgggagaag aagcaccggc taactacgtg ccagcagccg cggtaatacg
 481 tagggtgcaa gcgttgtccg gaattattgg gcgtaaagag ctcgtaggcg gtgtgtcacg
 541 tctgccgtga aaacctgcgg cttaaccgtg ggcgtgcggt ggatacgggc atcacttgag
 601 ttcggtaggg gagactggaa ttcctggtgt agcggtggaa tgcgcagata tcaggaggaa
 661 caccggtggc gaaggcgggt ctctgggccg atactgacgc tgaggagcga aagcgtgggg
 721 agcgaacagg attagatacc ctggtagtcc acgctgtaaa cgttgggcgc taggtgtggg
 781 gcgctgttca cgtgtcccgt gccgtagcta acgcattaag cgccccgcct ggggagtacg
 841 gccgcaaggc taaaactcaa aggaattgac gggggcccgc acaagcggcg gagcatgtgg
 901 attaattcga tgcaacgcga agaaccttac ctgggcttga catgcgccag acatccccag
 961 agatggggct tcccttgtgg ttggtgtaca ggtggtgcat ggctgtcgtc agctcgtgtc
1021 gtgagatgtt gggttaagtc ccgcaacgag cgcaaccctt gtcctatgtt gccagcgggt
1081 tatgccgggg actcgtggga gactgccggg gtcaactcgg aggaaggtgg ggatgacgtc
1141 aagtcatcat gcccccttatg tccagggctt cacacatgct acaatggctg gtacagaggg
1201 tggcgatgcc gtgaggtgga gcgaatccct taaagccggt ctcagttcgg atcgtagtct
1261 gcaactcgac tgcgtgaagt cggagtcgct agtaatcgca gatcagcagt gctgcggtga
1321 atacgttccc gggccttgta cacaccgccc gtcacgtcac gaaagtcggt aacacccgaa
1381 gcccatggcc caacctcttt cgggggggagg gagtggtcga aggtgggact ggcgattggg
1441 acgaagtcgt aacaaggtag ccgtaccgga aggtgcggct ggatcacctc ct
```

Saccharomonospora piscinae BCRC 16893

```
   1 agagtttgaa tcctggctca ggacgaacgc tggcggcgtg cttaacacat gcaagtcgaa
  61 cgctgaagct cagcttgctg ggtggatgag tggcgaacgg gtgagtaaca cgtgggtaat
 121 ctgccctgta ctctgggata agcccgggaa actgggtcta ataccggata ggacgcactc
 181 ctgcatgggg gtgtgtggaa agttccggcg gtacaggttg agcccgcggc ctatcagctt
 241 gttggtgggg tgatggccta ccaaggcgac gacgggtagc cggcctgaga gggtgaccgg
 301 ccacactggg actgagacac ggcccagact cctacgggag gcagcagtgg ggaatattgc
 361 acaatgggcg caagcctgat gcagcgacgc cgcgtggggg atgacggcct tcgggttgta
 421 aacccctttc gcccgggacg aagcgagagt gacggtaccg ggagaagaag caccggccaa
 481 ctacgtgcca gcagccgcgg taatacgtag ggtgcaagcg ttgtccggaa ttattgggcg
 541 taaagagctc gtaggcggtg tgtcacgtct gccgtgaaaa cctgcggctt aaccgtgggc
 601 gtgcggtgga tacgggcatc acttgagttc ggtaggggag actggaattc ctggtgtagc
 661 ggtggaatgc gcagatatca ggaggaacac cggtggcgaa ggcgggtctc tgggccgata
 721 ctgacgctga ggagcgaaag cgtggggagc gaacaggatt agataccctg gtagtccacg
 781 ccgtaaacgt tgggcgctag gtgtggggcg ctgttcacgt gtcccgtgcc gtagctaacg
 841 cattaagcgc cccgcctggg gagtacggcc gcaaggctaa aactcaaagg aattgacggg
 901 ggcccgcaca agcggcggag catgtggatt aattcgatgc aacgcgaaga accttacctg
```

961 ggcttgacat gcgccagaca gccccagaga tggggtttcc cttgtggttg gtgtacaggt
1021 ggtgcatggc tgtcgtcagc tcgtgtcgtg agatgttggg ttaagtcccg caacgagcgc
1081 aacccttgtc ccatgttgcc agcgggtaat gccggggact cgtgggagac tgccggggtc
1141 aactcggagg aaggtgggga tgacgtcaag tcatcatgcc ccttatgtcc agggcttcac
1201 acatgctaca atggccggta cagagggtag cgataccgtg aggtggagcg aatcccttaa
1261 agctggtctc agttcggatc gcagtctgca actcgactgc gtgaagtcgg agtcgctagt
1321 aatcgcagat cagcattgct gcggtgaata cgttcccggg ccttgtacac accgcccgtc
1381 acgtcatgaa agtcggtaac acccgaagcc catggcccaa ccagccttgt gctgggggga
1441 gtggttcgaa ggtggganct ggcgattggg acgaagt

Saccharomonospora saliphila YIM 90502

1 gattagagtt tgatcctggc tcaggacgaa cgctggcggc gtgcttaaca catgcaagtc
61 gaacgctgaa gctcagcttg ctgggtggat gagtggcgaa cgggtgagta acacgtgggt
121 aatctgccct gtactctggg ataagccctg gaaacggggt ctaataccgg atatgacatg
181 tctccgcatg ggggtgtgtg gaaagctccg gcggtacagg ttgagcccgc ggcctatcag
241 cttgttggtg gggtgatggc ctaccaaggc gacgacgggt agccggcctg agagggtgac
301 cggccacact gggactgaga cacggcccag actcctacgg gaggcagcag tggggaatat
361 tgcacaatgg gcgcaagcct gatgcagcga cgccgcgtgg gggatgacgg ccttcgggtt
421 gtaaacctct ttcgctaggg acgaagcctt tcggggtgac ggtacctgga gaagaagcac
481 cggctaacta cgtgccagca gccgcggtaa tacgtagggt gcgagcgttg tccggaatta
541 ttgggcgtaa agagctcgta ggcggtgtgt cgcgtctgcc gtgaaaacct gcggcttaac
601 cgtgggcgtg cggtggatac gggcatcact tgagttcggc aggggagact ggaattcctg
661 gtgtagcggt ggaatgcgca gatatcagga ggaacaccgg tggcgaaggc gggtctctgg
721 gccgatactg acgctgagga gcgaaagcgt ggggagcgaa caggattaga taccctggta
781 gtccacgccg taaacgttgg gcgctaggtg tgggacgctg ttcacgtgtc ccgtgccgta
841 gctaacgcat taagcgcccc gcctggggag tacggccgca aggctaaaac tcaaaggaat
901 tgacgggggc ccgcacaagc ggcggagcat gtggattaat tcgatgcaac gcgaagaacc
961 ttacctgggc ttgacatgca ccggatcgcc ccagagatgg ggtttccctt gtggctggtg
1021 tacaggtggt gcatggctgt cgtcagctcg tgtcgtgaga tgttgggtta agtcccgcaa
1081 cgagcgcaac ccttatccta tgttgccagc gggtaatgcc ggggactcgt gggagactgc
1141 cggggtcaac tcggaggaag gtggggatga cgtcaagtca tcatgccccc tatgtccagg
1201 gcttcacaca tgctacaatg gctggtacag agggtggcga taccgtgagg tggagcgaat
1261 cccttaaagc tggtctcagt tcggatcgta gtctgcaact cgactgcgtg aagtcggagt
1321 cgctagtaat cgcagatcag cagtgctgcg gtgaatacgt tcccgggcct tgtacacacc
1381 gcccgtcacg tcatgaaagt cggtaacacc cgaagcccgc ggcccaaccg ggttttcccg
1441 gggggagtgg tcgaaggtgg gactggcgat tgggacgaag tcgtaacaag gtagccgtac
1501 cggaaggtgc ggctggatca cctcctaat

Saccharomonospora viridis DSM 43017

1 ttgttggaga gtttgatcct ggctcaggac gaacgctggc ggcgtgctta acacatgcaa
61 gtcgaacgct gaagccgtct tcgggcggtg gatgagtggc gaacgggtga gtaacacgtg
121 ggtaatctgc cctgtactct gggataagcc tgggaaactg ggtctaatac cggataggac

```
 181 acgctaccgc atggtggtgt gtggaaagct tcggcggtac aggatgagcc cgcggcctat
 241 cagctagttg gtggggtgat ggcctaccaa ggcgacgacg ggtagccggc ctgagagggt
 301 gaccggccac actgggactg agacacggcc cagactccta cgggaggcag cagtggggaa
 361 tattgcacaa tgggcggaag cctgatgcag cgacgccgcg tgggggatga cggccttcgg
 421 gttgtaaacc cctttcgccc gggacgaagc gagagtgacg gtaccgggag aagaagcacc
 481 ggccaactac gtgccagcag ccgcggtaat acgtagggtg caagcgttgt ccggaattat
 541 tgggcgtaaa gagctcgtag gcggtgtgtc gcgtctgccg tgaaaacctg cggcttaacc
 601 gtgggcgtgc ggtggatacg ggcacacttg agttcggtag gggagactgg aattcctggt
 661 gtagcggtgg aatgcgcaga tatcaggagg aacaccagtg gcgaaggcgg gtctctgggc
 721 cgaaactgac gctgaggagc gaaagcgtgg ggagcgaaca ggattagata ccctggtagt
 781 ccacgccgta aacggtgggc gctaggtgtg ggatgctgtt cacgtgtccc gtgccgtagc
 841 taacgcatta agcgccccgc ctggggagta cggccgcaag gctaaaactc aaaggaattg
 901 acgggggccc gcacaagcgg cggagcatgt ggattaattc gatgcaacgc gaagaacctt
 961 acctgggttt gacatgcact ggaccggcgt agagatacgc cttcccttgt ggctggtgca
1021 caggtggtgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag tcccgcaacg
1081 agcgcaaccc ttgtcccatg ttgccagcgg gtaatgccgg ggactcgtgg gagactgccg
1141 gggtcaactc ggaggaaggt ggggacgacg tcaagtcatc atgcccctta tgcccagggc
1201 ttcacacatg ctacaatggc cagtacagag ggttgcgaga ccgtgaggtg gagcgaatcc
1261 cttaaagctg gtctcagttc ggatcgtagt ctgcaactcg actacgtgaa gtcggagtcg
1321 ctagtaatcg cagatcagca acgctgcggt gaatacgttc ccgggccttg tacacaccgc
1381 ccgtcacgtc atgaaagtcg gtaacacccg aagcccatgg cctaaccccg tcaggggagg
1441 gagtggtcga aggtgggacc ggcgattggg acgaagtcgt aacaaggtag ccgtaccgga
1501 aggtgcggct ggatcacctc cttt
```

Saccharomonospora xiaoerkulensis TRM 41495

```
   1 agtttgatcc tggctcagga cgaacgctgg cggcgtgctt aacacatgca agtcgaacgc
  61 tgaagcccag cttgctgggt ggaggagtgg cgaacgggtg agtaacacgt gggtaatctg
 121 ccctgtactc tgggataagc ctgggaaact gggtctaata ccggatagga catgctctcg
 181 catgagggtg tgtggaaagt tccggcggta caggttgagc ccgcggccta tcagcttgtt
 241 ggtggggtga tggcctacca aggcgacgac gggtagccgg cctgagaggg tgaccggcca
 301 cactgggact gagacacggc ccagactcct acgggaggca gcagtgggga atattgcaca
 361 atgggcgcaa gcctgatgca gcgacgccgc gtgggggatg acggccttcg ggttgtaaac
 421 ctctttcgcc cgggacgaag ggagactgac ggtaccggga gaagaagcac cggctaacta
 481 cgtgccagca gccgcggtaa tacgtagggt gcaagcgtcg tccggaatta ttgggcgtaa
 541 agagctcgta ggcggtgtgt tacgtctgcc gtgaaaacct gcggcttaac cgtgggcgtg
 601 cggtggatac gggcatcact tgagttcggc aggggagact ggaattcctg gtgtagcggt
 661 ggaatgcgca gatatcagga ggaacaccgg tggcgaaggc gggtctctgg gccgatactg
 721 acgctgagga gcgaaagcgt ggggagcgaa caggattaga taccctggta gtccacgccg
 781 taaacgttgg gcgctaggtg tggggcgctg ttcacgtgtc ccgtgccgta gctaacgcat
 841 taagcgcccc gcctggggga gtacggccgc aaggctaaaa actcaaagga attgacgggg
 901 ggcccgcaca agcggcggag catgtggatt aattcgatgc aacgcgaaga accttacctg
```

961 ggcttgacat gcaccggacg cgtccagaga tgggcgttcc cttgtggctg gtgtacaggt
1021 ggtgcatggc tgtcgtcagc tcgtgtcgtg agatgttggg ttaagtcccg caacgagcgc
1081 aacccttgtc ctatgttgcc agcgggtaat gccggggact cgtgggagac tgccggggtc
1141 aactcggagg aaggtgggga tgacgtcaag tcatcatgcc cctcatgtcc agggcttcac
1201 acatgctaca atggctggta cagagggtgg cgataccgtg aggtggagcg aatcccttaa
1261 agccggtctc agttcggatc gtagtctgca actcgactac gtgaagtcgg agtcgctagt
1321 aatcgcagat cagcagtgct gcggtgaata cgttcccggg ccttgtacac accgcccgtc
1381 acgtcatgaa agtcggtaac acccgaagcc cacggcccaa cccccgtgtg gggagggagt
1441 ggtcgaaggt gggactggcg attgggacga agtcgtaaca aggtagccga agggc

Saccharomonospora xinjiangensis XJ-54 = DSM 44391

1 cctggctcag gacgaacgct ggcggcgtgc ttaacacatg caagtcgaac gctgaagctc
61 agcttgctgg gtggatgagt ggcgaacggg tgagtaacac gtgggtaatc tgccctgtac
121 tctgggataa gcctgggaaa ctgggtctaa taccggatag gacacatcac cgcatggtgg
181 tgtgtggaaa gttccggcgg tacaggttga gcccgcggcc tatcagcttg ttggtggggt
241 gatggcctac caaggcgacg acgggtagcc ggcctgagag ggtgaccggc cacactggga
301 ctgagacacg gcccagactc ctacgggagg cagcagtggg gaatattgca caatgggcgc
361 aagcctgatg cagcgacgcc gcgtggggga tgacggcctt cgggttgtaa acccctttcg
421 cccgggacga agcgaaagtg acggtaccgg gagaagaagc accggccaac tacgtgccag
481 cagccgcggt aatacgtagg gtgcaagcgt tgtccggaat tattgggcgt aaagagctcg
541 taggcggtgt gtcacgtctg ccgtgaaaac ctgcggctta accgtgggcg tgcggtggat
601 acgggcatca cttgagttcg gtaggggaga ctggaattcc tggtgtagcg gtggaatgcg
661 cagatatcag gaggaacacc ggtggcgaag gcgggtctct gggccgaaac tgacgctgag
721 gagcgaaagc gtggggagcg aacaggatta gataccctgg tagtccacgc cgtaaacgtt
781 gggcgctagg tgtggggcgc tgttcacgtg tcccgtgccg tagctaacgc attaagcgcc
841 ccgcctgggg agtacggccg caaggctaaa actcaaagga attgacgggg gcccgcacaa
901 gcggcggagc atgtggatta attcgatgca acgcgaagaa ccttacctgg gcttgacatg
961 catcagacga ctccagagat ggggtttccc ttgtggctgg tgcacaggtg gtgcatggct
1021 gtcgtcagct cgtgtcgtga gatgttgggt taagtcccgc aacgagcgca acccttgtcc
1081 catgttgcca gcgggtaatg ccggggactc gtgggagact gccggggtca actcggagga
1141 aggtggggat gacgtcaagt catcatgccc cttatgtcca gggcttcaca catgctacaa
1201 tggctggtac agagggttgc gataccgtga ggtggagcga atcccttaaa gccagtctca
1261 gttcggatcg cagtctgcaa ctcgactgcg tgaagtcgga gtcgctagta atcgcagatc
1321 agcattgctg cggtgaatac gttcccgggc cttgtacaca ccgcccgtca cgtcatgaaa
1381 gtcggtaaca cccgaagccc atggcccaac ccttcgggga gggagtggtc gaaggtggga
1441 ctggcgattg ggacgaagtc gtaacaaggt agccgtaccg gaaggtgcgg

Actinopolyspora algeriensis H19

1 gtttgatcct ggctcaggac gaacgctgac ggcgcgcttc acacatgcaa gtcgaacgct
61 cgcaccccgt gtggctcttt tcgaagggtt ggggtgtggg agtggcggac gggtgagtaa
121 cacgtgagta acctgccccg ggcgtgggga taactccggg aaactggggc taataccgga
181 tgtgctgcat gcctcgcatg gggtgtgtgg gaaaggttca tycytgtgag ggggtgttcc

241 ggcctgggtg gggctcgcgg cccatcagct tgttggtgcg gtgagggcgt accaaggcga
301 tgacgggtag ccggcctgag agggtgatcg gccacactgg gactgagaca cggcccagac
361 tcctacggga ggcagcagtg gggaattttg cgcaatgggc gaaagcctga cgcagcgacg
421 ccgtgtgggg gaggacggcc ttcgggttgt aaaccccttt cggccctgac gaatgtgacg
481 gtaggggcta aagaagcgcc ggctaactac gtgccagcag ccgcggtaat acgtacggcg
541 cgagcgttgt ccggatttac tgggcgtaaa gggctcgtag gcggtttgtc gcgtcggtcg
601 tggaaatgcg cagctcaact gggcacgtgc ggctgatacg ggcagactcg agggcggtag
661 gggcaagcgg aattcctggt gtagcggtga aatgcgcaga tatcaggagg aacaccgatg
721 gcgaaggcag cttgctgggc cgttcctgac gctgaggagc gaaagcatgg gtagcgaaca
781 ggattagata ccctggtagt ccatgctgta aacgttgggc gctaggtgtg gggaccgttg
841 tggtgtccgt gccgtagcta acgcattaag cgccccgcct ggggagtacg gccgcaaggc
901 taaaactcaa aggaattgac gggggcccgc acaagcggcg gagcatgtgg attaattcga
961 tgcaacgcga agaaccttac ctgggtttga catacaccgg attgcctcag agatggggtt
1021 tcccttgtgg ctggtgtaca ggtggtgcat ggctgtcgtc agctcgtgtc gtgagatgtt
1081 gggttaagtc ccgtaacgag cgcaaccctt gtcctgtgtt gccagcggtt cggccgggga
1141 ctcgcgggag actgccgggg tcaactcgga ggaaggcggg gacgacgtca agtcatcatg
1201 ccccttatgt ccagggcttc acacatgcta caatggccgg tacagagggt ggcgagaccg
1261 tgaggtggag cgaatcccgg aaagccggtc tcagttcgga tcggggtctg caactcgacc
1321 ctgtgaagtc ggagtcgcta gtaatcgcag atcagcaacg ctgcggtgaa tacgttcccg
1381 ggccttgtac acaccgcccg tcacgtcatg aaagtcggta acaccctaag ctcatggtcc
1441 aaccacacgg tgtgtggggg gcgtggtcga aggtgggact ggcgattggg acgaagtcgt
1501 aacaaggtag ccgtaccgga aggtgcggct ggatcacctc cttt

Annexe III

Similarity matrix calculated by *PAST* 4 based on *Jaccard*'s coefficient.

Similarity and distance indices

	S. piscinae	S. amisosen	S. azurea	S. colocasia	S. cyanea	S. glauca	S. halophila	S. iraqiensis	S. iraqiensis	S. marina	S. oceani	S. saliphila	S. viridis	S. xiaoerkul	S. xinjiange
S. piscinae	1	0.26415094	0.26530612	0.35897436	0.21428571	0.30952381	0.43396226	0.38095238	0.325	0.41304348	0.55	0.36363636	0.33333333	0.51351351	0.26829268
S. amisosen	0.26415094	1	0.48888889	0.41463415	0.47368421	0.39534884	0.39655172	0.28571429	0.28888889	0.52173913	0.39583333	0.31578947	0.35416667	0.35555556	0.35714286
S. azurea	0.26530612	0.48888889	1	0.43243243	0.54545455	0.41025641	0.28813559	0.26086957	0.26190476	0.41304348	0.29166667	0.32352941	0.53846154	0.36585366	0.36842105
S. colocasia	0.35897436	0.41463415	0.43243243	1	0.44827586	0.5862069	0.31372549	0.28947368	0.33333333	0.43589744	0.325	0.33333333	0.45714286	0.38235294	0.26470588
S. cyanea	0.21428571	0.47368421	0.54545455	0.44827586	1	0.46666667	0.2745098	0.20512821	0.27272727	0.38461538	0.21428571	0.30769231	0.48484848	0.32352941	0.4137931
S. glauca	0.30952381	0.39534884	0.41025641	0.5862069	0.46666667	1	0.38	0.275	0.39393939	0.41463415	0.34146341	0.35714286	0.51428571	0.36111111	0.25
S. halophila	0.43396226	0.39655172	0.28813559	0.31372549	0.2745098	0.38	1	0.38461538	0.34	0.41071429	0.40740741	0.2826087	0.34545455	0.42857143	0.29411765
S. iraqiensis	0.38095238	0.28571429	0.26086957	0.28947368	0.20512821	0.275	0.38461538	1	0.32432432	0.29787234	0.41463415	0.36666667	0.30232558	0.44444444	0.23076923
S. iraqiensis	0.325	0.28888889	0.26190476	0.33333333	0.27272727	0.39393939	0.34	0.32432432	1	0.4	0.325	0.44	0.34210526	0.46875	0.22857143
S. marina	0.41304348	0.52173913	0.41304348	0.43589744	0.38461538	0.41463415	0.41071429	0.29787234	0.4	1	0.58536585	0.37142857	0.36956522	0.51282051	0.27906977
S. oceani	0.55	0.39583333	0.29166667	0.325	0.21428571	0.34146341	0.40740741	0.41463415	0.325	0.58536585	1	0.4516129	0.30434783	0.47368421	0.23809524
S. saliphila	0.36363636	0.31578947	0.32352941	0.33333333	0.30769231	0.35714286	0.2826087	0.36666667	0.44	0.37142857	0.4516129	1	0.34375	0.56	0.2962963
S. viridis	0.33333333	0.35416667	0.53846154	0.45714286	0.48484848	0.51428571	0.34545455	0.30232558	0.34210526	0.36956522	0.30434783	0.34375	1	0.42105263	0.25
S. xiaoerkul	0.51351351	0.35555556	0.36585366	0.38235294	0.32352941	0.36111111	0.42857143	0.44444444	0.46875	0.51282051	0.47368421	0.56	0.42105263	1	0.4375
S. xinjiange	0.26829268	0.35714286	0.36842105	0.26470588	0.4137931	0.25	0.29411765	0.23076923	0.22857143	0.27906977	0.23809524	0.2962963	0.25	0.4375	1

Similarity matrix calculated by *PAST* 4 based on *Kulczynski-2*'s coefficient.

Similarity and distance indices

	S. piscinae	S. amisosen	S. azurea	S. colocasia	S. cyanea	S. glauca	S. halophila	S. iraqiensis	S. iraqiensis	S. marina	S. oceani	S. saliphila	S. viridis	S. xiaoerkul	S. xinjiange
S. piscinae	1	0.4202509	0.41935484	0.54398827	0.37016129	0.48051075	0.6265233	0.55436081	0.50513196	0.58586338	0.70967742	0.62211982	0.50055617	0.68645161	0.43932412
S. amisosen	0.4202509	1	0.66039427	0.62247475	0.7	0.59027778	0.575	0.4537037	0.4760101	0.68627451	0.5703405	0.5952381	0.52921456	0.54222222	0.56547619
S. azurea	0.41935484	0.66039427	1	0.62170088	0.74032258	0.59139785	0.46308244	0.41577061	0.42741935	0.58586338	0.4516129	0.5702765	0.70077864	0.54193548	0.55913978
S. colocasia	0.54398827	0.62247475	0.62170088	1	0.62045455	0.7405303	0.54141414	0.4537037	0.5	0.63636364	0.50513196	0.52597403	0.63949843	0.55545455	0.41883117
S. cyanea	0.37016129	0.7	0.74032258	0.62045455	1	0.64166667	0.50555556	0.34814815	0.42954545	0.59558824	0.37016129	0.48571429	0.67586207	0.495	0.58571429
S. glauca	0.48051075	0.59027778	0.59139785	0.7405303	0.64166667	1	0.60694444	0.43287037	0.56628788	0.60416667	0.51747312	0.56547619	0.68534483	0.53083333	0.40178571
S. halophila	0.6265233	0.575	0.46308244	0.54141414	0.50555556	0.60694444	1	0.59259259	0.57525253	0.59379085	0.59928315	0.60873016	0.53869732	0.65333333	0.52380952
S. iraqiensis	0.55436081	0.4537037	0.41577061	0.4537037	0.34814815	0.43287037	0.59259259	1	0.49494949	0.46514161	0.58900836	0.59656085	0.46487867	0.6162963	0.38095238
S. iraqiensis	0.50513196	0.4760101	0.42741935	0.5	0.42954545	0.56628788	0.57525253	0.49494949	1	0.59893048	0.50513196	0.64285714	0.51959248	0.64090909	0.37229437
S. marina	0.58586338	0.68627451	0.58586338	0.63636364	0.59558824	0.60416667	0.59379085	0.46514161	0.59893048	1	0.74003795	0.65546218	0.54310345	0.69411765	0.46218487
S. oceani	0.70967742	0.5703405	0.4516129	0.50513196	0.37016129	0.51747312	0.59928315	0.58900836	0.50513196	0.74003795	1	0.72580645	0.46718576	0.65032258	0.39938556
S. saliphila	0.62211982	0.5952381	0.5702765	0.52597403	0.48571429	0.56547619	0.60873016	0.59656085	0.64285714	0.65546218	0.72580645	1	0.58251232	0.78	0.47619048
S. viridis	0.50055617	0.52921456	0.70077864	0.63949843	0.67586207	0.68534483	0.53869732	0.46487867	0.51959248	0.54310345	0.46718576	0.58251232	1	0.59586207	0.41050903
S. xiaoerkul	0.68645161	0.54222222	0.54193548	0.55545455	0.495	0.53083333	0.65333333	0.6162963	0.64090909	0.69411765	0.65032258	0.78	0.59586207	1	0.61333333
S. xinjiange	0.43932412	0.56547619	0.55913978	0.41883117	0.58571429	0.40178571	0.52380952	0.38095238	0.37229437	0.46218487	0.39938556	0.47619048	0.41050903	0.61333333	1

Annexe IV

Globale chemotaxonomy matrix of *Saccahraomonosporae*

Species	GLU	MAN	GAL	RIB	ARA	XYL	MAD	Un S	Glu	Ala	Gly
S. piscinae	1	1	1	1	1	0	1	0	0	0	0
S. amisosensis	1	0	1	0	1	1	0	0	1	1	0
S. azurea	0	0	1	0	1	0	0	0	1	1	0
S. colocasiae	1	0	1	1	1	0	0	0	0	0	0
S. cyanea	0	0	1	0	1	0	0	0	1	1	0
S. glauca	0	0	1	0	1	0	0	0	0	0	0
S. halophila	1	1	1	1	1	0	0	1	0	0	0
S. iraqiensis subsp. *iraqiensis*	0	0	1	1	1	0	0	0	0	0	0
S. iraqiensis subsp. *paurometabolica*	0	0	1	1	1	0	0	0	0	0	0
S. marina	0	0	1	1	1	0	0	0	0	0	0
S. oceani	1	0	1	0	1	0	0	0	0	0	0
S. saliphila	0	0	1	0	1	0	0	0	0	0	0
S. viridis	0	1	1	1	1	0	0	0	1	1	1
S. xiaoerkulensis	0	1	1	1	1	0	0	0	0	0	0
S. xinjiangensis	0	0	1	0	1	0	0	0	1	1	0

Globale chemotaxonomy matrix of *Saccahraomonosporae* -Continued-

Species	NGlu	LL-DAP	meso-DAP	Mur	N Mur	MK07 (H2)	MK07 (H4)	MK08(H2)	MK08 (H4)
S. piscinae	0	0	1	0	0	0	0	0	1
S. amisosensis	1	0	1	1	0	0	1	0	1
S. azurea	1	0	1	1	0	0	0	0	1
S. colocasiae	0	0	1	0	1	0	0	0	1
S. cyanea	1	0	1	0	0	0	0	0	1
S. glauca	0	0	1	0	0	0	0	0	1
S. halophila	1	0	1	0	0	0	0	0	1
S. iraqiensis subsp. *iraqiensis*	0	1	1	0	0	0	0	1	1
S. iraqiensis subsp. *paurometabolica*	0	0	1	0	0	0	0	0	0
S. marina	0	0	1	0	0	0	0	0	1
S. oceani	0	0	1	0	0	0	0	0	1
S. saliphila	0	0	1	0	0	0	0	0	1
S. viridis	0	0	1	0	0	0	0	0	1
S. xiaoerkulensis	0	0	1	0	0	0	0	0	1
S. xinjiangensis	1	0	1	1	0	1	1	0	1

Globale chemotaxonomy matrix of *Saccahraomonosporae* -Continued-

Species	MK08 (H6)	MK09 (H2)	MK09 (H4)	MK09 (H6)	MK010 (H4)	PE	PI	PG	PIM	DPG
S. piscinae	0	0	1	1	0	1	0	1	0	1
S. amisosensis	0	0	1	0	0	1	1	0	1	1
S. azurea	0	0	1	0	0	0	1	1	0	1
S. colocasiae	0	0	1	0	0	1	0	0	0	1
S. cyanea	0	0	1	0	0	0	0	0	0	0
S. glauca	0	0	1	0	0	1	0	0	0	0
S. halophila	1	0	1	1	0	1	1	0	0	1
S. iraqiensis subsp. *iraqiensis*	1	1	1	0	1	1	1	1	1	1
S. iraqiensis subsp. *paurometabolica*	0	1	1	0	0	1	1	1	0	1
S. marina	0	0	1	0	0	1	1	1	0	1
S. oceani	0	0	1	0	0	1	1	1	1	1
S. saliphila	0	0	1	0	0	1	1	1	0	1
S. viridis	1	0	1	1	0	0	1	1	0	0
S. xiaoerkulensis	0	1	1	0	0	1	1	1	0	1
S. xinjiangensis	0	1	1	0	0	1	0	0	0	0

Globale chemotaxonomy matrix of *Saccahraomonosporae* -Continued-

Species	GPL	GL	PME	LPE	LPG	PL (U)	HPE	NPG	AL	AP
S. piscinae	0	0	1	0	0	1	1	1	0	0
S. amisosensis	0	0	0	0	0	0	0	0	1	1
S. azurea	0	1	0	0	0	1	0	1	0	0
S. colocasiae	0	0	0	0	0	0	0	0	0	0
S. cyanea	0	0	0	0	0	0	0	0	0	0
S. glauca	0	0	0	1	0	0	1	0	0	0
S. halophila	0	0	1	1	0	1	1	0	0	0
S. iraqiensis subsp. *iraqiensis*	0	0	1	0	1	1	0	0	0	0
S. iraqiensis subsp. *paurometabolica*	0	0	0	0	0	0	1	0	0	0
S. marina	0	0	0	0	0	0	0	0	0	0
S. oceani	0	0	1	0	0	1	0	0	0	0
S. saliphila	0	0	0	0	0	0	0	0	0	0
S. viridis	0	1	0	0	0	0	0	1	0	0
S. xiaoerkulensis	0	0	0	0	0	1	0	0	0	0
S. xinjiangensis	0	0	0	0	0	1	0	0	0	0

Globale chemotaxonomy matrix of *Saccahraomonosporae* -Continued-

Species	$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	$C_{14:1}$ *w5c*	$C_{15:0\ 2\ OH}$	$C_{15:1}$ B	$C_{15:1}$ *w6c*
S. piscinae	0	0	0	0	0	0	0	0	0	1
S. amisosensis	0	1	1	1	1	1	0	0	1	0
S. azurea	0	1	1	1	1	0	0	1	1	0
S. colocasiae	0	0	1	1	0	0	0	1	1	0
S. cyanea	0	0	1	1	1	0	0	0	0	0
S. glauca	0	0	1	1	0	1	0	1	0	0
S. halophila	1	1	1	1	1	1	0	0	0	0
S. iraqiensis subsp. *iraqiensis*	0	1	0	0	0	0	0	0	0	0
S. iraqiensis subsp. *paurometabolica*	0	0	0	1	0	1	0	0	0	0
S. marina	0	1	1	1	1	1	1	0	1	1
S. oceani	0	1	0	0	0	0	1	0	0	1
S. saliphila	0	0	0	0	0	0	0	0	0	0
S. viridis	0	0	1	1	0	0	0	1	0	0
S. xiaoerkulensis	0	0	0	1	1	0	0	0	0	0
S. xinjiangensis	0	0	1	1	1	0	0	0	0	0

Globale chemotaxonomy matrix of *Saccahraomonosporae* -Continued-

Species	$C_{16:0\ 2\ OH}$	$C_{16:1}$	$C_{16:1}$ *cis* 9	$C_{16:1}$ *w6c*	$C_{16:1}$ *w7c*	$C_{16:1}$ *w9c*	$C_{17:0}$ *w8c*	$C_{17:1}$	$C_{17:1}$ *w6c*	$C_{17:1}$ *cis* 9
S. piscinae	0	0	0	1	1	0	1	0	1	0
S. amisosensis	0	0	1	0	0	0	0	0	0	1
S. azurea	0	0	1	0	0	1	0	0	0	0
S. colocasiae	0	0	0	0	0	0	0	0	0	0
S. cyanea	0	0	1	0	0	0	0	0	0	1
S. glauca	0	0	0	0	0	1	0	0	0	0
S. halophila	1	1	0	0	0	0	0	1	1	0
S. iraqiensis subsp. *iraqiensis*	0	0	0	0	0	0	0	0	0	0
S. iraqiensis subsp. *paurometabolica*	0	1	0	0	0	0	0	0	0	0
S. marina	0	0	1	1	0	0	0	0	1	1
S. oceani	0	0	0	1	1	0	0	0	1	0
S. saliphila	0	0	0	0	0	0	0	0	1	0
S. viridis	0	0	0	0	0	0	0	0	0	0
S. xiaoerkulensis	0	0	0	0	0	0	0	0	1	0
S. xinjiangensis	0	0	0	0	0	0	0	0	1	0

Globale chemotaxonomy matrix of *Saccahraomonosporae* -Continued-

Species	$C_{17:1}$ *w*8c	$C_{17:1}$ *w*9*c*	$C_{18:1}$	$C_{18:1}$ *cis* 9	$C_{18:1}$ *w*9c	iso-$C_{14:0}$	iso-$C_{15:0}$	anteiso-$C_{15:0}$	anteiso-$C_{15:0\ 2\ OH}$	iso-$C_{16:0}$
S. piscinae	1	0	0	0	0	1	1	0	0	1
S. amisosensis	0	0	0	1	0	1	1	0	0	1
S. azurea	0	1	0	0	0	0	1	1	0	1
S. colocasiae	0	1	0	0	0	1	1	1	0	1
S. cyanea	0	1	0	0	0	0	1	1	0	1
S. glauca	0	1	0	0	0	1	1	1	1	1
S. halophila	1	0	0	1	0	1	1	1	1	1
S. iraqiensis subsp. *iraqiensis*	0	0	0	0	0	1	0	1	0	0
S. iraqiensis subsp. *paurometabolica*	0	0	1	0	0	0	1	0	1	1
S. marina	1	0	0	0	1	1	1	0	1	1
S. oceani	1	0	0	0	0	1	1	0	1	1
S. saliphila	0	0	0	0	0	0	1	0	0	1
S. viridis	0	1	0	0	0	0	1	1	1	1
S. xiaoerkulensis	1	0	0	0	1	0	1	0	0	1
S. xinjiangensis	1	0	0	0	0	0	0	0	0	1

Globale chemotaxonomy matrix of *Saccahraomonosporae* -Continued-

Species	anteiso-$C_{16:0}$	iso-$C_{16:0\ 2\ OH}$	iso-$C_{16:1}$	iso-$C_{16:1\ H}$	anteiso-$C_{16:0}$	iso-$C_{17:0}$	iso-$C_{17:0\ 2\text{-}OH}$
S. piscinae	0	0	0	1	1	1	0
S. amisosensis	0	1	0	1	0	1	0
S. azurea	1	1	0	1	0	1	0
S. colocasiae	0	1	0	1	0	0	0
S. cyanea	0	1	0	0	0	1	0
S. glauca	0	1	0	1	0	1	0
S. halophila	0	0	0	0	1	1	1
S. iraqiensis subsp. *iraqiensis*	0	0	0	0	1	1	0
S. iraqiensis subsp. *paurometabolica*	0	0	1	0	0	1	0
S. marina	0	1	0	1	0	1	0
S. oceani	0	0	0	1	0	1	0
S. saliphila	0	0	0	0	0	1	0
S. viridis	0	1	0	1	0	1	0
S. xiaoerkulensis	0	0	0	1	0	1	0
S. xinjiangensis	0	0	0	0	0	0	0

Globale chemotaxonomy matrix of *Saccahraomonosporae* -Continued-

Species	iso-$C_{17:1}$	iso-$C_{17:1}$ w9c	anteis-$C_{17:0}$	anteiso-$C_{17:0\ 2OH}$	anteiso-$C_{17:1\ C}$	iso-$C_{18:0}$	10 Methyl $C_{16:0}$	10 Methyl $C_{17:0}$
S. piscinae	0	0	1	0	0	0	0	0
S. amisosensis	0	0	1	0	0	1	1	0
S. azurea	0	0	0	0	0	0	0	0
S. colocasiae	0	0	1	0	0	0	0	0
S. cyanea	0	0	1	0	0	0	0	0
S. glauca	0	0	1	0	0	1	0	0
S. halophila	0	0	1	1	1	1	1	0
S. iraqiensis subsp. *iraqiensis*	0	0	1	0	0	1	0	0
S. iraqiensis subsp. *paurometabolica*	1	0	1	0	0	0	0	0
S. marina	0	0	1	0	0	0	1	1
S. oceani	0	1	1	0	0	1	1	1
S. saliphila	0	0	0	0	0	1	0	0
S. viridis	0	0	1	0	0	1	0	0
S. xiaoerkulensis	0	0	1	0	0	1	0	0
S. xinjiangensis	0	0	0	0	0	0	0	0