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on *Jaccard* and *Dice* coefficients) of the genus *Kineococcus***

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**Molecular and chemotaxonomic comparison study (based on *Jaccard* and
Dice coefficients) of the genus *Kineococcus***

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ملخص

تم إجراء دراسة كيموتصنيفية (كيميائية-تصنيفية)، وكذلك دراسة جزيئية على خمسة عشر نوعاً من البكتيريا الهيفية التي تنتمي إلى جنس *Kineococcus*، وهي:

K. aurantiacus, *K. aureolus*, *K. endophyticus*, *K. glutinatus*, *K. gynurae*, *K. gypseus*,
K. mangrovi, *K. radiotolerans*, *K. rhizosphaerae*, *K. rubinsiae*, *K. siccus*, *K. terrestris*,
K. vitellinus *K. indalonis* و *K. xinjiangensis* .

يهدف هذا العمل إلى إنشاء مقارنة كيموتصنيفية (مبنية على التركيب الكيميائي الخلوي على أساس معاملي *Jaccard* و *Dice* الإحصائيين) بين الأنواع المدروسة ومقارنة جزيئية (مبنية على تحليل مورثة الـ ARNr 16S وكذلك على تحليل الجينوم بكامله) من خلال تحديد أقرب الأنواع إلى نوع مرجعي مختار هو *K. vitellinus* وفقاً لدرجة التشابه، والمسافة التطورية. إذ تمّ استخدام المعاملين الإحصائيين، *Jaccard* و *Dice*، لإجراء التحليل الكيميائي، باستخدام المعلومات التي تم جمعها في المؤلفات العلمية المتعلقة بالتكوين الخلوي في تحديد وتصنيف الأنواع المدروسة. تستخدم الدراسة الجزيئية خوارزميات المعلوماتية الحيوية لمحاذاة تسلسل النوكليوتيدات.

نستخلص من الدراسة أنه بالرغم من الحصول على ترتيب تقارب متطابق بين الطريقة الجزيئية والطريقة الكيميائية بالمعاملين *Jaccard* و *Dice*، إلا أنه يستحسن أخذ نتائج الدراسة الكيميائية عموماً بتحفظ لما فيها من نقائص ولكون الدراسة الجزيئية أفضل لأجل تمييز الأنواع داخل الجنس الواحد، ومع الاستعانة بفحص الجينوم الكلي والمعامل ANI لأجل تجنّب حالات الالتباس بين الأنواع ذات التقارب التصنيفي الشديد.

الكلمات المفتاحية: *Kineococcus*، دراسة تصنيفية كيميائية، معامل *Jaccard*، معامل *Dice*، الدراسة

الجزيئية، *Kineococcus vitellinus*.

Résumé

Une étude comparative entre les méthodes chimiotaxonomique (étude de taxonomie chimique) et les méthodes de taxonomie moléculaire a été réalisée sur quinze espèces de *actinobactéries* appartenant au genre *Kineococcus*, qui sont :

K. aurantiacus, *K. aureolus*, *K. endophyticus*, *K. glutinatus*, *K. gynurae*, *K. gypseus*, *K. mangrovi*, *K. radiotolerans*, *K. xinjiangensis*, *K. rhizosphaerae*, *K. rubinsiae*, *K. siccus*, *K. terrestris*, *K. vitellinus* et *K. indalonis*.

Ce travail vise à établir une comparaison chimiotaxonomique (basée sur la composition chimique cellulaire) par les coefficients statistiques de *Jaccard* et *Dice* entre les espèces étudiées et une comparaison moléculaire (basée sur l'analyse du gène de l'ARNr 16S ainsi que sur l'analyse du génome entier) en identifiant l'espèce la plus proche d'une espèce de référence choisie *K. vitellinus* selon le degré de similarité et la distance évolutive. Les coefficients statistiques *Jaccard* et *Dice* ont été utilisés pour effectuer une analyse chimique en utilisant les informations recueillies dans la littérature scientifique relative à la composition cellulaire pour identifier et classer les espèces étudiées. L'étude moléculaire utilise des algorithmes bioinformatiques pour aligner les séquences nucléotidiques.

Nous concluons de l'étude que même si un ordre de proximité identique a été obtenu entre la méthode moléculaire et la méthode chimique avec les coefficients de *Jaccard* et *Dice*, il est préférable de prendre les résultats de l'étude chimique en général avec prudence en raison de ses lacunes et le fait que l'étude moléculaire est plus efficace pour distinguer les espèces au sein d'un même genre, à l'aide de l'analyse du génome entier et du coefficient ANI, afin d'éviter toute confusion entre les espèces à forte affinité taxonomique.

Mots clés : *Kineococcus*, étude chimiotaxonomique, coefficient de *Jaccard*, coefficient de *Dice*, étude moléculaire, *Kineococcus vitellinus*.

Abstract

A comparative study between a chemotaxonomy method (chemical taxonomy study) and a molecular taxonomy method was conducted on fifteen species of *actinobacteria* belonging to the genus *Kineococcus*, which are:

K. aurantiacus, *K. aureolus*, *K. endophyticus*, *K. glutinatus*, *K. gynurae*, *K. gypseus*, *K. mangrovi*, *K. radiotolerans*, *K. xinjiangensis*, *K. rhizosphaerae*, *K. rubinsiae*, *K. siccus*, *K. terrestris*, *K. vitellinus* and *K. indalonis*.

This work aims to establish a chemotaxonomic comparison (based on cellular chemical composition) by *Jaccard* and *Dice* statistical coefficients between the studied species and a molecular comparison (based on analysis of the 16S rRNA gene as well as on analysis of the entire genome) by identifying the closest species to a chosen reference specie.

K. vitellinus according to degree of similarity and evolutionary distance. The statistical coefficients *Jaccard* and *Dice*, were used to conduct a chemical analysis using information collected from the scientific literature related to cellular composition to identify and classify the studied species. The molecular study uses bioinformatics algorithms to align nucleotide sequences.

We conclude from the study that although an identical order of closeness ranking was obtained between the molecular method and the chemical method with the *Jaccard* and *Dice* coefficients, it is preferable to take the results of the chemical study in general with caution due to its shortcomings and the fact that the molecular study is better for distinguishing species within a single genus, with the help of whole genome analysis and the ANI coefficient, in order to avoid confusion between species with high taxonomic affinity.

Keywords: *Kineococcus*, chemotaxonomcal study, *Jaccard* coefficient, *Dice* coefficient, molecular study, *Kineococcus vitellinus*.

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List of abbreviations

DAP: Diaminopimelic Acid

rRNA: Ribosomal Ribonucleic Acid

DNA: Deoxyribonucleic Acid

DDH: DNA-DNA Hybridization.

dDDH: Digital DNA-DNA Hybridization.

FASTA: FAST-All

G+C: Guanine + Cytosine

MEGA: Molecular Evolutionary Genetics Analysis

MK: Menaquinone

MR: Methyl Red

VP: Voges-Proskauer

NJ: Neighbor Joining

PAST: PAleontological STatistics

ANI: Average Nucleotide Identity

AHC: Agglomerative Hierarchical Clustering

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INTRODUCTION

INTRODUCTION

The ancient phylum *Actinobacteria* (homotypic synonyms: *Actinomycetota*) is composed of phylogenetically and physiologically diverse bacteria that help Earth's ecosystem function. as free-living organisms and symbionts of herbivorous. *Actinobacteria* are ubiquitous and one of the most diverse groups of bacteria in nature. Its members range from anaerobic to aerobic, from unicellular organisms to filamentous, and spore-forming lineages strains. They are Gram-positive bacteria with high G+C DNA content (Barka *et al.*, 2016).

They also have an extensive secondary metabolism and produce a large and excessive number of bioactive natural products relevant for agriculture, biotechnology, and medicine, including the majority of the antibiotics that we use, as well as many anticancer, anthelmintic, and antifungal compounds (Van der Meij *et al.*, 2017). *Actinobacteria* are not only the main producers of microbial-derived drugs; they also play an important role as symbionts in plant-associated microbial communities (Barka *et al.*, 2016).

The community structure, diversity, biological activities, and mechanisms of environmental adaptation of those *Actinobacteria* in special and extreme environments are comparatively unknown and unclear when compared to those from ordinary habitats. Their functions and utilization are even less well reported (Qin *et al.*, 2019).

A distinctive characteristic of this bacterial group is its capacity to utilize a diverse array of substrates present in soil (Schleifer, 2009).

Kineococcus is one of the genera within the *Actinobacteria* group. It was specifically selected for a comparative analysis through chemotaxonomic methods (including the *Jaccard* index and the *Dice* index) and molecular methods (utilizing 16S rRNA sequences and whole-genome sequencing). The study involved analyzing 15 species within the genus and creating dendrograms to visualize their similarity and relationship to *K. vitellinus*, which served as the reference species. The primary objective of this comparison was to assess whether the simpler taxonomic analyses could serve as alternatives to the more complex phylogenetic and phylogenomic studies in determining the genetic closeness or distance of species in relation to a specific reference species.

CHAPTER I: BIBLIOGRAPHIC REVIEW

CHAPTER I: Bibliographic review

1. Actinobacteria

1.1 An introduction to *Actinobacteria*

Actinobacteria cause confusion when we make comparisons with other bacteria and act like fungi due to their morphological characteristics, but they have a high concentration of G+C (from 55% to more than 70%). The phylum is very diverse phenotypically and morphologically (from cocci to mycelium with significant differentiation). Species are aerobic, anaerobic, or facultatively anaerobic (Goodfellow *et al.*, 2012).

Actinobacteria have a wide metabolic range, with a strong preference for chemoorganotrophs, and they are instrumental in the composting of biomaterials, particularly slowly degrading biomaterials such as cellulose, Chitin and lignin contribute to the formation of humus in the soil. (Ranjani *et al.*, 2016).

The economic and industrial value of *Actinobacteria* is undisputed due to their well-known ability to produce primary and secondary metabolites (Subramani and Sipkema, 2019). *Actinobacteria* have been identified as producing 13,700 bioactive molecules, including anti-tumor, immunomodulator, phytohormones, pesticides, and antibiotics. *Actinobacteria* have promising potential to be a biocontrol tool for plant pathogens due to their antagonistic activities (Ranjani *et al.*, 2016).

1.2 Systematic of *Actinobacteria*

The phylum *Actinobacteria* is one of the largest taxonomic units among the major lineages currently recognized within the Bacteria domain (Gao and Gupta, 2012). The classification of *Actinobacteria* has evolved, guided by advances in molecular techniques. While 16S rRNA analysis remains a widely used method for *Actinobacteria* identification, the phylum *Actinobacteria* at the highest level is now divided into six classes, namely, *Actinobacteridae*, *Acidimicrobidae*, *Coriobacteridae*, *Nitriliruptoridae*, *Thermoleophilia* and *Rubrobacteridae*. These subclasses are further subdivided into a number of different orders and suborders in Figure 1. It is noteworthy that in this taxonomy, 46 orders and 79 families within the phylum *Actinobacteria* are part of a single subclass, *Actinobacteridae*, whereas the other four subclasses together contained only 10 families (Barka *et al.*, 2016).

Kineococcus is a genus within the family *Kineosporiaceae*. The type strain is *Kineococcus aurantiacus* (Yokota *et al.*, 1993). The classification of *K. aurantiacus* is as follows:

Domain: *Bacteria*

Phylum: *Actinobacteria*

Class: *Actinomycetia*

Order: *Kineosporiales*

Family: *Kineosporiaceae*

Genus: *Kineococcus*

Type species: *Kineococcus aurantiacus* (Yokota *et al.*, 1993).

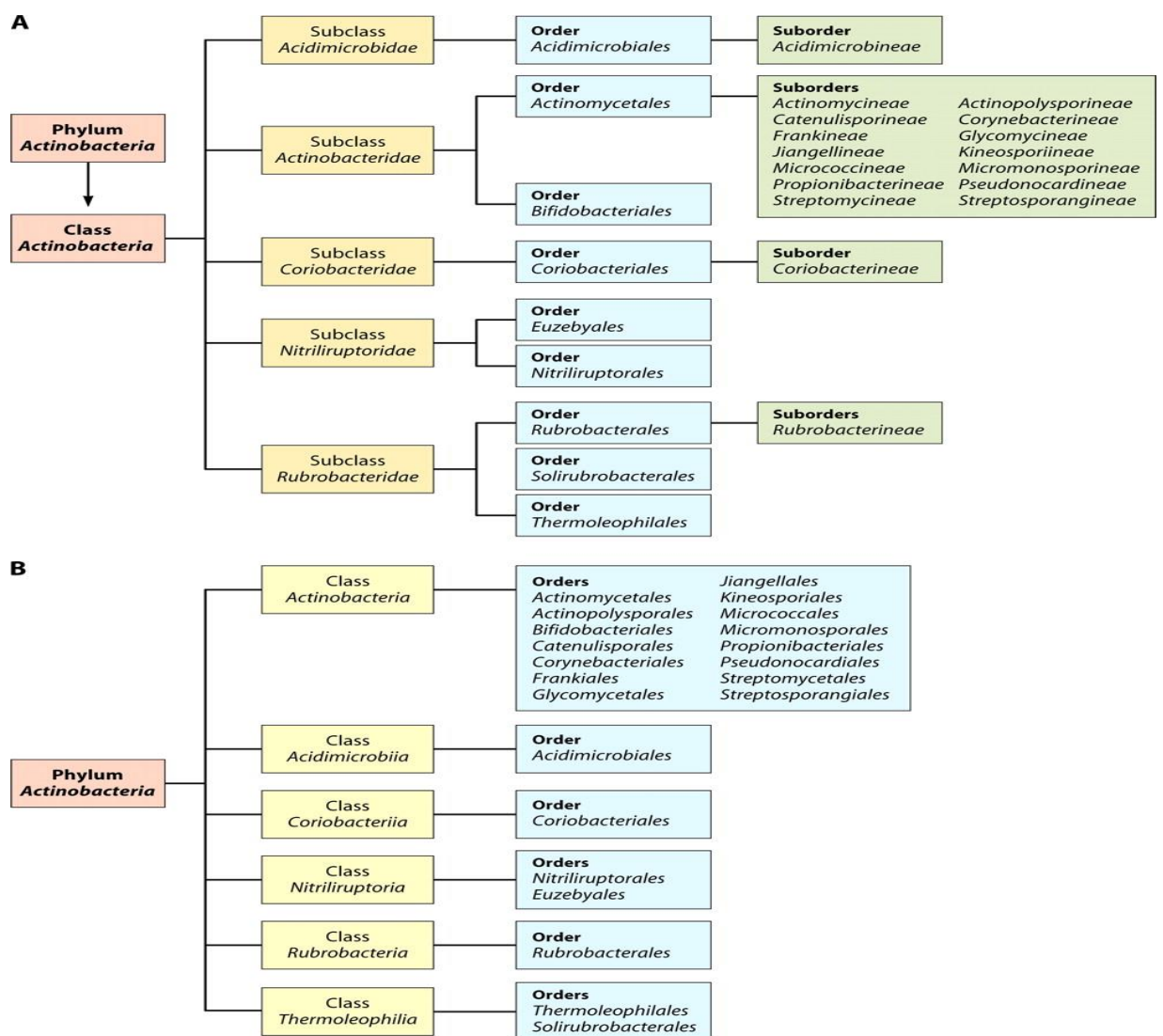


Figure 1 Current taxonomic outline for the phylum *Actinobacteria* (A) and proposed taxonomy for *Actinobacteria* in the upcoming bergey's manual of systematic bacteriology (Gao and Gupta, 2012).

1.3 Taxonomy of Actinobacteria

Taxonomy is the science of the classification of various living organisms consisting of three independent, but interrelated disciplines, namely classification, nomenclature and identification (Gajdács *et al.*, 2019).

Modern bacterial nomenclature is rooted in the binomial system proposed by the 18th century naturalist Carl Linnaeus, who established the concept of taxonomy based on a genus and species. Initially, genus and species names were derived from visualized organism characteristics or the names of the individuals who first described or discovered the organism (Schleifer, 2009).

Later, after growing organisms from culture in the laboratory was well established, Bergey's first edition of determinative bacteriology systematically classified bacteria into hierarchies based on distinguishing phenotypic characteristics (Gajdács *et al.*, 2019).

Although practical, phenotypic classification could not provide an evolutionary framework to elucidate organisms relatedness, which led to an explosion of molecular and genetic testing methodologies aimed at improving the taxonomy and classification in the following years (Prinzi *et al.*, 2023).

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 analyze 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 dendrograms revealed 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 (Vishakha *et al.*, 2019).

1.3.1 Phenotypic taxonomy

Phenotype was also used for decades to classify microorganisms despite much less conspicuous morphological and developmental traits than animals and plants. However, phenotype provides little insight into deep evolutionary relationships of microorganisms, which can only be discerned by comparison of conserved information-bearing macromolecule (Hugenholtz *et al.*, 2021).

Phenotypic characteristics of bacteria comprise morphological, physiological, and biochemical features. Individually, many of these characteristics have been shown to be irrelevant as parameters for genetic relatedness, yet as a whole, they provide descriptive information enabling us to recognize taxa (Vandamme *et al.*, 1996). The morphology of a

bacterium includes both cellular (shape, endospore, flagella, Gram staining) and colonial (color, dimensions, form) characteristics. The physiological and biochemical features include data on growth at different temperatures, pH, salt concentrations, or atmospheric conditions, growth in the presence of various substances such as antimicrobial agents, and data on the presence or activity of various enzymes, metabolization of compounds, etc.

1.3.2 Morphological identification

The description of the morphology is very important at the genus level. Cultural, macromorphological and micromorphological characters are assessed according to « Bergey's Manual » of 1989 and 1994 (Boudjelal-Bencheikh, 2012).

- **Macromorphology**

The morphology and growth characteristics of *Actinobacteria* in diverse types of cultural media are referred to as their cultural features. By comparing the colors of the substrate, aerial mycelia, and any soluble pigments with chips from the ISCC-NBS color charts, the colors of the samples were verified (Li *et al.*, 2016).

The Figure 2 shows the scanning electron microscope of *Kineococcus rubinsiae*.

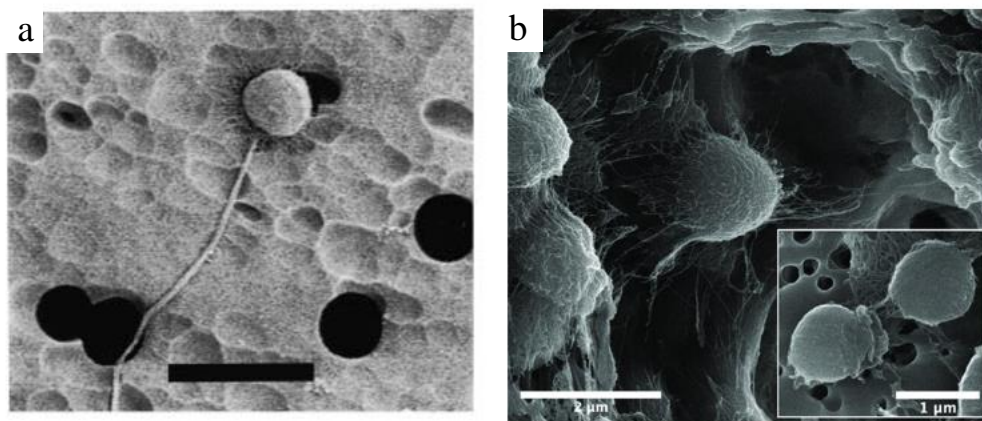


Figure 2. Scanning electron microscopy of *Kineococcus rubinsiae* (Mhatre *et al.*, 2021).

- **Micromorphology**

The matrix of mycelium, often referred to as vegetative mycelium, grows into or on top of the culture medium. The primary role of the substrate mycelium is to take up nutrients so that *Actinobacteria* can thrive. The substrate mycelia seem thinner, transparent and more branching under a microscope than aerial mycelium have a thickness of around 0.4 to 1.2 μm , are capable of branching out, and often do not form diaphragms or break. Minority groups

(like *Nocardia*) that are simple to highly branching like roots and substrate hyphae may break in place or under mechanical disruption into coccoid to rod-shaped, not-motile parts (Li *et al.*, 2016).

Micrography of some *Kineococcus* species are shown in Figure 3 bellow.

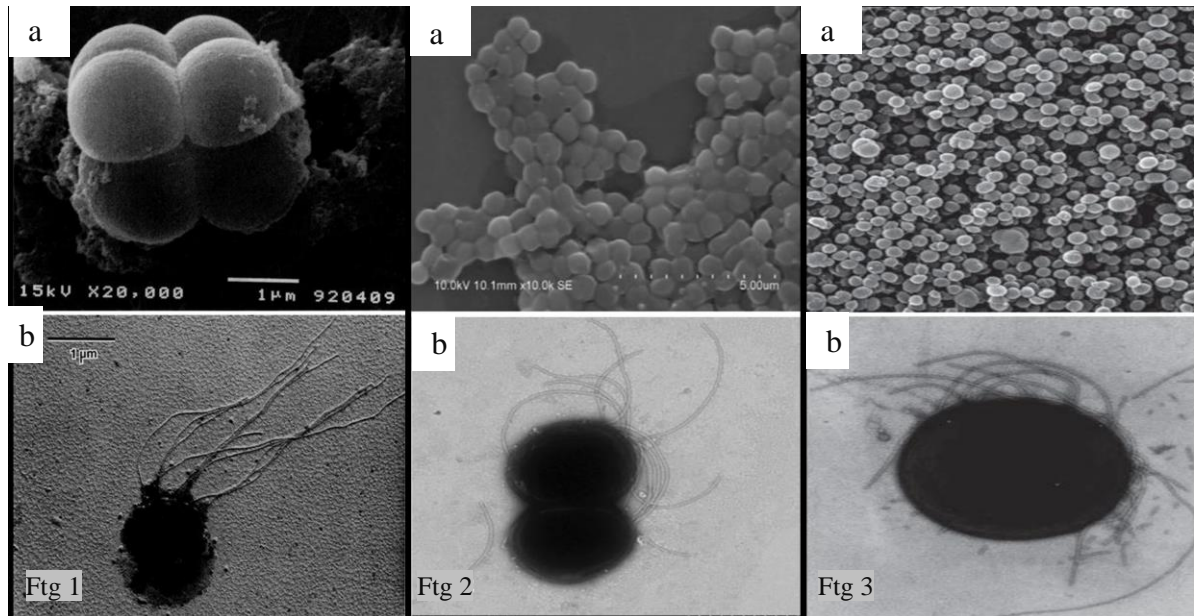


Figure 3. Micrography of some *Kineococcus* species.

Fig 1(a) Scanning electron micrograph of *Kineococcus aurantiacus*. Fig 1 (b) Transmission electron micrograph of a motile cell of *Kineococcus aurantiacus* (Yokota *et al.*, 1993).

Fig 2(a) Scanning electron micrograph of *Kineococcus endophyticus*. Fig 2 (b) Transmission electron micrograph of a motile cell of *Kineococcus endophyticus* (Bian *et al.*, 2012).

Fig 3(a) Scanning electron micrograph of *Kineococcus magrovi*. Fig 3 (b) Transmission electron micrograph of a motile cell of *Kineococcus magrovi* (Duangmal *et al.*, 2016).

- **Physiological and biochemical identification**

The activity of bacterial enzymes and protein regulators is closely linked with physiological and biochemical properties. Since proteins and enzymes are products of genes, comparing the physiological and biochemical features of *Actinobacteria* is an indirect comparison of their genomes, and determining their physiological and biochemical properties is much simpler than analyzing the genome directly (Li *et al.*, 2016).

Many aspects need to be taken into account in order for physiological and biochemical test findings to be meaningful. The 16S rRNA studies should be used to compare the new strain to other related strains and closely related strains. Because phenotypic features can be influenced by a wide range of parameters, including cultural conditions, a strict and ideally

standardized methodology is required to obtain results that are comparable to those of earlier research. Duplicate or triple testing is also necessary (Xu *et al.*, 2007).

A novel species' description can be assembled using the polyphasic methodology and the thorough findings of multiple techniques, including morphological, physiological, rRNA gene sequencing and chemotaxonomic indicators. As many of the physiological and biochemical features of a new species are unpredictable even with a whole genome sequence (Li *et al.*, 2016). The features and their variations among groups are shown in Table 1.

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

Features	The variations among groups
Temperature adaptability	The ideal temperature for growth and the minimum, maximum, and ideal temperature for death
pH values adaptability	The pH range within which it can grow and the ideal pH for growth
Using of nitrogen source	Usage of amino acids, proteins, nitrogen, peptone, inorganic salt, N ₂ , etc.
Using of carbon source and acid-producing ability	Using of different alcohols, organic acids, polysaccharides, disaccharides, monosaccharides and carbohydrate-based acid generation.
Growth factors	Vitamins, amino acids.
Atmospheric condition	Aerobic, microaerophilic, anaerobic, facultative anaerobic
Antimicrobial activity against pathogens	Inhibition of filamentous fungus, yeast, and both Gram-positive and Gram-negative bacteria, etc.
Metabolization	Different characteristic metabolites tests, such as MR test, VP test, iodole production, etc.
Different enzymes Activity	Oxidase, catalase, urease, etc.
Susceptibility	The susceptibility to antimicrobial substances, antibiotics, etc...

1.3.3 Chemotaxonomy of *Actinobacteria*

The scientific area of chemotaxonomy classifies organisms based on their cell wall composition (polysaccharides (sugars), peptidoglycan cell walls, amino acids, fatty acids, and phospholipids) (Zitouni *et al.*, 2005).

Whereby, cell wall macromolecules of prokaryote microorganisms were some of the first chemotaxonomic markers to be used for prokaryote taxonomic research. However, these

observations were not possible until the development of methods such as electron microscopy and chromatography, which allowed the separation and purification of cell wall material (Cummins and Harris, 1956; Salton, 1994).

Depending on the species and genus, the makeup of these components determines the genus of *Actinobacteria*. This method works well for genera that have a significant diversity in chemical composition but share morphological or genetic characteristics.

The taxonomy of *Actinobacteria* groups becomes easier with the analysis of the composition of their cell walls, which aids in differentiating between genus and species. (Barka *et al.*, 2015).

1.3.3.1 Chemotaxonomic biomarkers

Organic chemical researchers were the ones who initially employed chemotaxonomy to study naturally occurring compounds in plants. Many botanists and chemists discovered they could identify and classify plants based on their chemical composition as natural product chemistry advanced (Fang *et al.*, 2024). Many of the chemical macromolecules that are now the basis of chemotaxonomic biomarkers, which are widely used to detect and categorize microorganisms, were first identified in the early 1900s. These early studies demonstrated that different kinds of bacteria have different components in their cell membranes (Salton, 1994). Chemotaxonomic markers and their location within the cell are shown in the Table 2 below.

Table 2. Cellular locations of chemotaxonomic markers (Xu *et al.*, 2007).

Chemotaxonomic markers	Location within the cell
Amino acids	The cell wall
Sugars	The whole cell
Menaquinones	The plasmic membrane
Polar lipids	The plasmic membrane
Fatty acids	The plasmic membrane
Mycolic acid	The plasmic membrane

1.3.3.1.1 Amino acid and peptidoglycan composition

It is common practice to use the amino acid composition of peptides as a critical phenotypic characteristic for *Actinobacteria* species identification. Actinobacterial peptides usually consist of D-isoglutamine, L-alanine, L-diamino acid, and D-alanine, where various

lineages differ in the third position of the L-diamino acid. For instance (Petit *et al.*, 1969; Kato *et al.*, 1968).

Peptidoglycan is a heteropolymer consisting of glycan branches that are often cross-linked by short peptides and consist of alternating β -1,4-linked *N*-acetylglucosamine and *N*-acetyl muramic acid residues with different cell membrane contents (Ghuysen, 1968; Vollmer *et al.*, 2008).

The structures of some amino acids that can be found in cell membrane are shown in Figure 4.

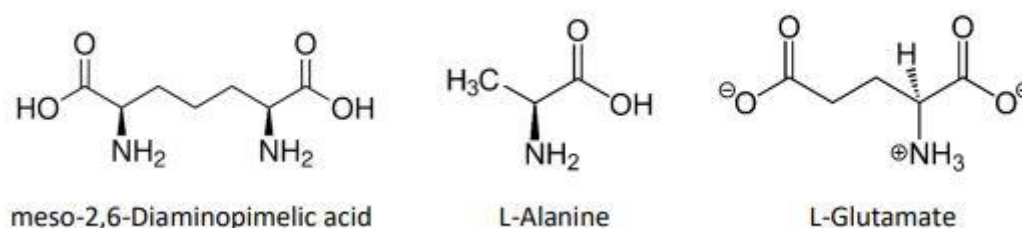


Figure 4. Cell wall chemotypes in *Actinobacteria* (Larpent, 2000).

1.3.3.1.2 Whole cell sugar composition

The whole cell sugar analysis is very important in the classification and identification of *Actinobacteria* (Lechevalier, 1968). *Actinobacteria* can be divided into five characteristic chemotypes depending on the presence of some characteristic sugars in Table 3 (Lechevalier and Lechevalier, 1980). 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 *Actinobacteria* (Lechevalier and Lechevalier, 1980).

Table 3. Sugar chemotypes in *Actinobacteria* (Lechevalier and Lechevalier, 1970 ; Labeda and Lechevalier, 1989).

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

1.3.3.1.3 Lipid composition

Lipid profile is of major importance for the classification of *Actinobacteria*, as the amino acid and sugar composition can be insufficient for the identification and classification in many genera of *Actinobacteria*. 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).

1.3.3.1.4 Phospholipids

Variations exist in the membrane composition of various species of bacteria, and even within a single species, the composition of the membrane varies depending on the external conditions that the cells are exposed to a wide variety of amphiphilic lipids can be found in the membranes of bacteria, such as the less common phospholipids phosphatidylcholine and phosphatidylinositol, as well as the most common phospholipids phosphatidylglycerol, phosphatidylethanolamine, and cardiolipin (Sohlenkamp and Geiger, 2016).

One of the most significant features of phospholipids in the cell is the structure of their membrane. It enables the majority of cells with the same chemotype to be distinguished (Lechevalier *et al.*, 1977).

Phospholipid chemotypes in *Actinobacteria* are shown in Table 4.

Table 4. Phospholipid chemotypes in *Actinobacteria* (lechevalier *et al.*, 1977).

Type	Types of phospholipids	Exemples
PI	PG	<i>Actinomadura</i>
PII	PE	<i>Streptomyces, Pseudonocardia</i>
PIII	PC	<i>Nocardiosis, Amycolatopsis</i>
PIV	PL + GluA + PE	<i>Nocardia, Nonomuraea</i>
PV	PG + PL + GLUA	<i>Oerskovia</i>

PG: Phosphatidylglycerol, PE: Phosphatidylethanolamine, PC: Phosphatidylcholine, PL: Phospholipids, GluA: Glucosamine.

1.3.3.1.5 Fatty acids

The majority of fatty acids found in *Actinobacteria* are classified as either mycolic acids, which have 20 to 90 carbon atoms, or as molecules with 12 to 20 carbon atoms.

Rhodococcus, *Mycobacterium*, and *Nocardia* are the common genera that are known to contain mycolic acids (Harir *et al.*, 2018).

The chemical structures of some fatty acids, including saturated fatty acids, are shown in Figure 5.

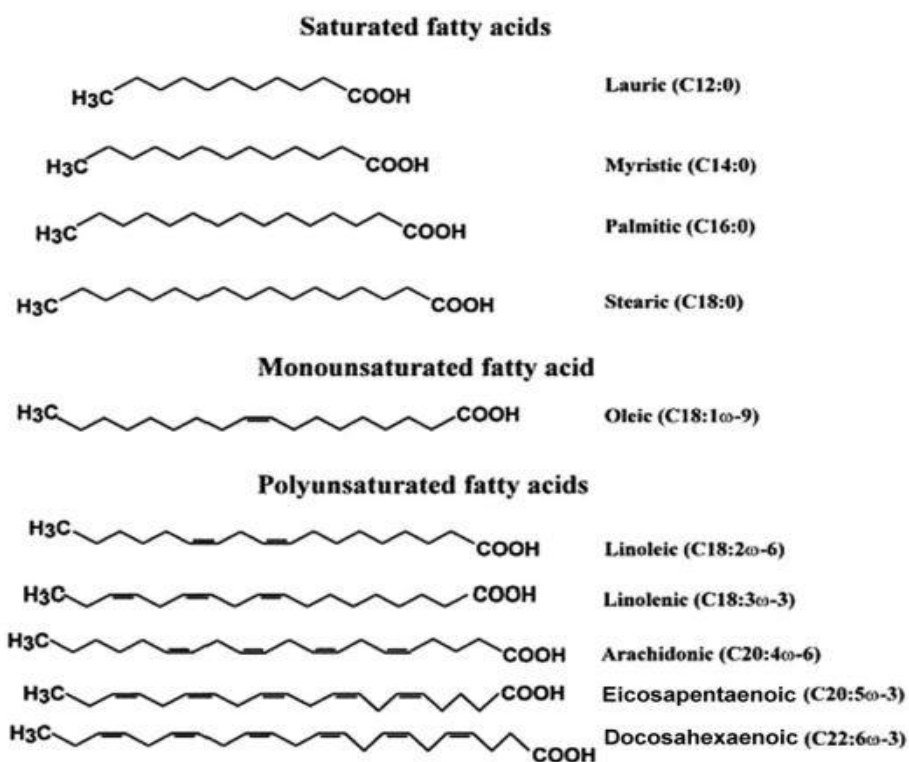


Figure 5. Chemical structures of some fatty acids (Grenon *et al.*, 2012).

Fatty acids profile in *Actinobacteria* are shown in Table 5.

Table 5. Fatty acids profile in *Actinobacteria* (Kroppenstedt and Eventushenko, 2006).

Type	Branched chain fatty acids							Cyclo propane
	Saturated	Unsaturated	Iso 14/16/18	Iso 15/17	Anteiso 15/17	10-Methyl		
						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%).

1.3.3.1.6 Menaquinones

Microorganisms inhabit vastly different environments and have different lifestyles, but most of them used ATP as an energy source. These microorganisms used different respiratory processes with different sets of electron transport mechanisms.

Menaquinones patterns of *Actinobacteria* is shown in Table 6. Menaquinones are soluble lipids that are necessary for respiration and can be found at the plasmatic membrane. They participate in the phosphate oxidation and electron transport processes. A methylated naphthoquinone nucleus (2-methyl-1-4-naphthoquinone) connected to an isoprene side chain form their structure. They are distinguished based on the quantity of isoprene units and the degree of chain hydrogenation (Minnikin *et al.*, 1984; Rodríguez Concepción and Boronat, 2013). They move electrons across various respiratory chain enzymes, which is how they contribute significantly to bacterial respiration. Menaquinones can also be used by *Actinobacteria* as a source of precursors for the manufacture of certain metabolites, like pigments and antibiotics. Thus, menaquinones are essential for the development and metabolism of *actinobacteria*.

Table 6. Menaquinones patterns of *Actinobacteria* (Williams *et al.*, 1983).

Family	Major menaquinones
<i>Actinomycetaceae</i>	MK-9
	MK-10(H ₄)
	MK-9(H ₄)
	MK-8
<i>Actinospicaceae</i>	MK-9(H ₆)
	MK-9(H ₄)
	MK-9(H ₈)
<i>Beutenbergiaceae</i>	MK-8(H ₄)
<i>Nocardoidaceae</i>	MK-8(H ₄)
	MK9(H ₆)
	MK-10(H ₆)
<i>Kineosporiaceae</i>	MK-9(H ₄)
	MK-9(H ₂), or MK-8(H ₂)

1.3.4 Phylogenetic taxonomy

It is commonly accepted that chemotaxonomic biomarkers are quite stable within different prokaryote taxa. However, with the advancements in genomes and proteomics, the applicability of these traditional chemotaxonomic markers to contemporary taxonomy is being questioned (Sutcliffe *et al.*, 2013; Mahato *et al.*, 2017).

Differences between laboratories subjectivity in data interpretation, repeated use of reference strains/materials (which increases costs), and lack of transferable/searchable databases could influence the generation of accurate information and the exchange of related knowledge (Whitman, 2014). These reasons generally impelled the move away from traditional chemotaxonomic methodology to genomic and phylogenomic taxonomy (Sutcliffe *et al.*, 2013, Whitman, 2014).

Actinobacteria has in its DNA a high concentration of guanine and cytosine among the 18 primary lineages that have recently been classified under the domain of bacteria.

The division of the phylum *Actinobacteria* relies on the location of its branches in 16S rRNA gene trees. Nevertheless, uncertainty could happen since 16S rRNA sequences have poor discrimination between closely related species and some genera. For example, the taxonomic classification of the *Kitasatospora* genus (Omura *et al.*, 1982). Recently, the new taxonomy of the phylum *Actinobacteria* based on 16S rRNA trees was published. The previous subclasses and suborders were reorganized into the ranks of classes and orders (Gao and Gupta., 2012).

1.3.4.1 Study of ribosomal RNA (16S rRNA)

The field of microbial studies has advanced revolutionarily as a result of the creation and widespread use of culture-independent molecular technologies. Determining conserved regions in the sequence reference is the first step towards determining the variable regions. The improvement of these technologies makes a substantial contribution to the study of microorganisms that are not detectable through conventional techniques like culture in dishes methods. all the molecular techniques aimed at the 16S rRNA gene. Recent investigations employing next-generation sequencing (NGS) technologies have demonstrated that a greater number of bacteria and taxa previously believed to inhabit different parts of the human body and different locations on Earth (Fukuda *et al.*, 2016).

The schematic of the ribosome complex and 16S rRNA gene is shown in Figure 6 bellow.

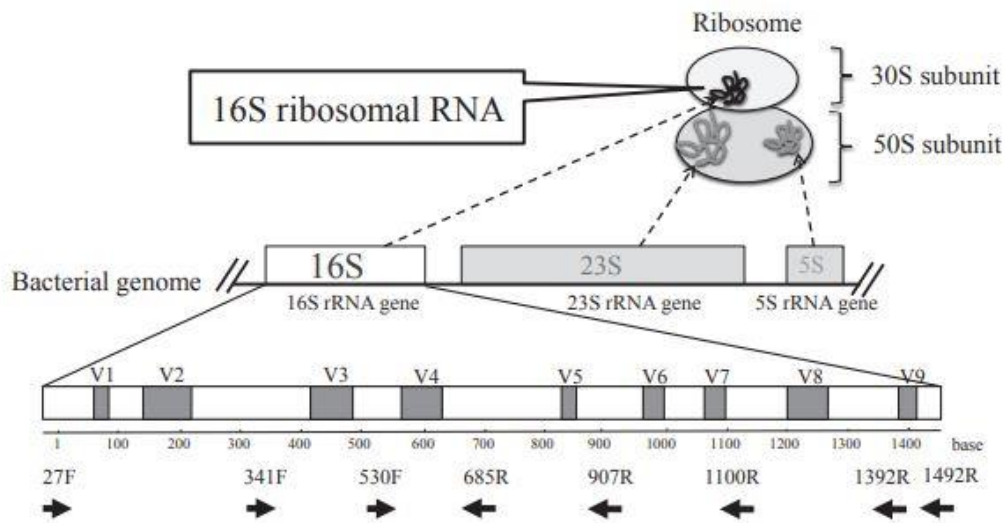


Figure 6. Illustration of the ribosome complex and 16S rRNA gene. White boxes indicate conserved regions, and gray boxes indicate hypervariable regions. The approximate locations of universal primers on the 16S rRNA gene are indicated by the bold arrows (Fukuda *et al.*, 2016).

1.3.4.2 DNA-DNA hybridization (DDH)

DNA–DNA hybridization (DDH) techniques, also known as DNA–DNA reassociation techniques, have been used as the gold standard for the genomic similarity analyses of pairwise sets of strains for classification purposes. The method has had an enormous relevance during the last half a century of classification of prokaryotes, are based on an attempt to make raw comparisons of whole genomes between different organisms in order to calculate their overall genomic similarities (Richter *et al.*, 2009).

DNA–DNA hybridization (DDH) experiments have been performed to determine relatedness between bacteria, as this was one of the few universally applicable techniques available that could offer truly genome-wide comparisons between organisms. A value of 70% “the gold standard”. DDH was proposed by Wayne *et al.* (1987) as a recommended standard for delineating species (Goris *et al.*, 2007). The necessity for bacterial identification arises when the similarity value of 16S rRNA between two strains exceeds 98.65% (Vandamme *et al.*, 1996; Kim *et al.*, 2014). However, due to the labor-intensive and error-prone nature of DDH experiments, there has been an ongoing demand for alternative genotype-based standards such as average nucleotide identity (ANI) and digital DDH

(dDDH), computed using recommended settings of the Genome-to-Genome Distance Calculator (GGDC), among others (Kim *et al.*, 2014; Chen *et al.*, 2016).

1.3.4.3 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 come 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 for G+C content are 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 (Goodfellow *et al.*, 2018).

1.4 Habitat and ecology of *Actinobacteria*

Within the bacterial community, the *Actinobacteria* phylum occupies the biggest taxonomic aspects, functions, and compounds. They are currently known as bacteria because their genomes have large concentrations of G+C (Garrity *et al.*, 2004; Sun *et al.*, 2010). While many researchers have described plants associated with *Actinobacteria*, many environmental conditions may also have an impact on the diversity and species distribution within the host plant (Bouizgarne and Aouamar, 2014; Hou *et al.*, 2009).

- **Soil habitat**

Actinobacteria perform well in vegetative growth in low-humidity environments, although their growth can be inhibited in dry soils with a lack of moisture (Barka *et al.*, 2016). *Actinobacteria* grow best in environments with a pH between 6.0 and 9.0. Since pH 7.0 was the point at which their growth reached its peak, they exhibit neutrophil behavior. However, *Actinobacteria*, particularly those of the genus *Streptomyces*, cannot grow in an environment with a pH of less than 3.5 (Kim *et al.*, 2003).

- **Plants habitat**

The primary and abundant home of endophytic *Actinomycetota* with high biodiversity is tropical rainforests. An essential factor in this interaction is how the plant reacts to its endophyte. Finding out if endophytic communities benefit plants or rhizospheric bacteria

more has always been an intriguing field of study. On the other hand, a diverse range of microbial endophytes inhabit the endosphere of plants, creating a sophisticated micro-ecosystem (El-Shatoury *et al.*, 2013).

- **Marine habitat**

Marine *Actinobacteria* with abundant cellulolytic activity have been discovered in marine habitats (Magarvey *et al.*, 2004). Many researchers have reported on the distribution of *Actinobacteria* in the marine environment. Because marine bacteria can produce significant organic metabolites, they can also be a valuable subject for research (Bull and Stach., 2007). The habitat diversity of *Actinobacteria* in extreme environments is shown in Figure 7.

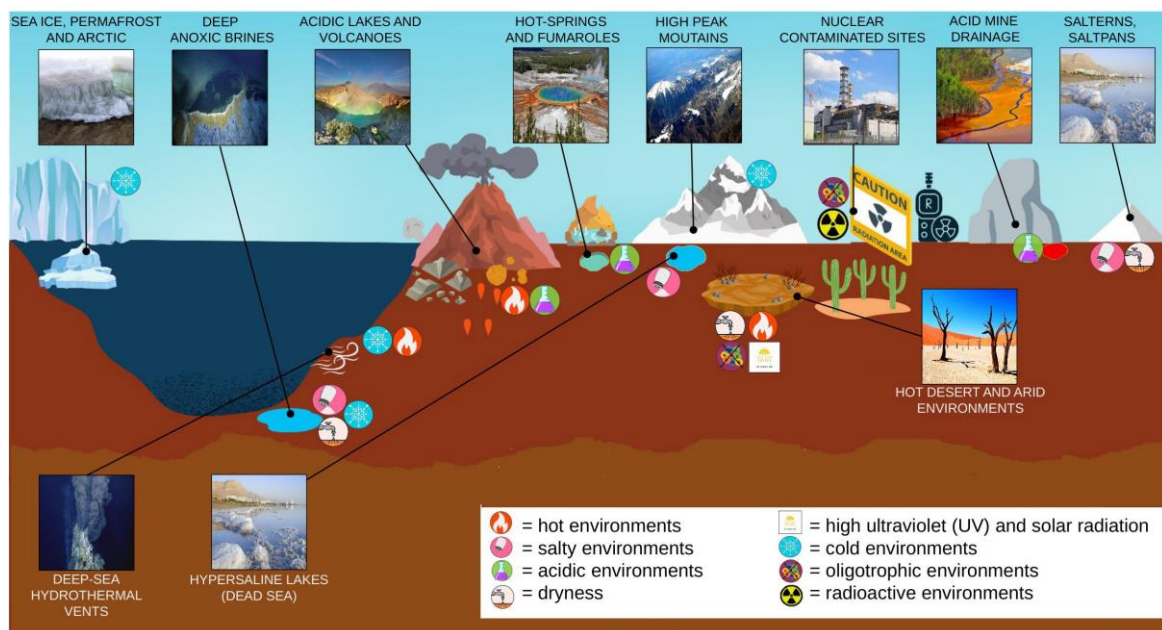


Figure 7. The habitat diversity of *Actinobacteria* in extreme environments (Coleine *et al.*, 2022).

The diversity and abundance of *actinobacteria* in different ecosystems Table 7 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 use, pollution, and antibiotic use can also have significant impacts on *Actinomycetota* communities and their ecological roles (Ranjani *et al.*, 2016).

Table 7. Ecological distribution of *Actinobacteria* (Goel *et al.*, 2021).

Actinobacterial strain	Type of habitat	Habitat
<i>Rubrobacter</i> sp. <i>Amycolatopsis</i> sp. <i>Thermobifida</i> sp. <i>Thermotunica</i> sp. <i>Saccharomonospora</i> sp. <i>Georgenia</i> sp.	Extreme	Extreme environment
<i>Dietzia</i> sp. <i>Agrococcus</i> sp. <i>Arthrobacter</i> sp. <i>Gordonia</i> sp. <i>Mycobacterium</i> sp. <i>Pseudonocardia</i> sp. <i>Rhodococci</i> sp. <i>Streptomyces</i> sp.	Marine	Aquatic environment
<i>Actinoplanes</i> sp. <i>Micromonospora</i> sp. <i>Rhodococcus</i> sp. <i>Streptomyces</i> sp. <i>Thermoactinomyces</i> sp.	Freshwater	
<i>Streptomyces</i> sp. <i>Nocardia</i> sp. <i>Streptoverticillium</i> sp. <i>Nocardiopsis</i> sp. <i>Amycolatopsis</i> sp. <i>Micromonospora</i> sp. <i>Actinomadura</i> sp.	Soil	Terrestrial environment

1.5 Genus of *Kineococcus*

Yokota *et al.* (1993) suggested the genus *Kineococcus*, which belongs to the *Kineosporiaceae* family and has 15 confirmed species: *Kineococcus aurantiacus* (Yokota *et al.*, 1993), *K. radiotolerans* (Phillips *et al.*, 2002), *K. gynurae* (Duangmal *et al.*, 2008), *K. rhizosphaerae* (Lee, 2009), *K. xinjiangensis* (Liu *et al.*, 2009), *K. glutinatus* (Nie *et al.*, 2012), *K. endophyticus* (Bian *et al.*, 2012), *K. gypseus* (Li *et al.*, 2015), *K. mangrovi* (Duangmal *et al.*, 2016), *K. terrestris* (Xu *et al.*, 2017), *K. aureolus* (Xu *et al.*, 2017), *K. rubinsiae* (Mhatre *et al.*, 2021), *K. vitellinus* (Molina-Menor *et al.*, 2021), *K. indalonis* (Molina-Menor *et al.*, 2021), and *K. siccus* (Molina-Menor *et al.*, 2021).

The incapacity for spore formation sets *Kineococcus* apart from other members of the *Kineosporiaceae* family. With the exception of three species (*K. vitellinus*, *K. indalonis*, and *K. siccus*), due to an insufficient amount of data, the cell wall pattern contains meso-DAP acid and galactose, Anteiso C_{15:0} is the primary fatty acid, and MK9 (H₂) is the primary

menaquinone as a chemotaxonomic marker. The characteristic phospholipids are diphosphatidylglycerol and phosphatidylglycerol, with the exception of *K. gynurae* and *K. xinjiangensis*.

The polyphasic approach in identifying *Actinomycetota* are shown in Figure 8.

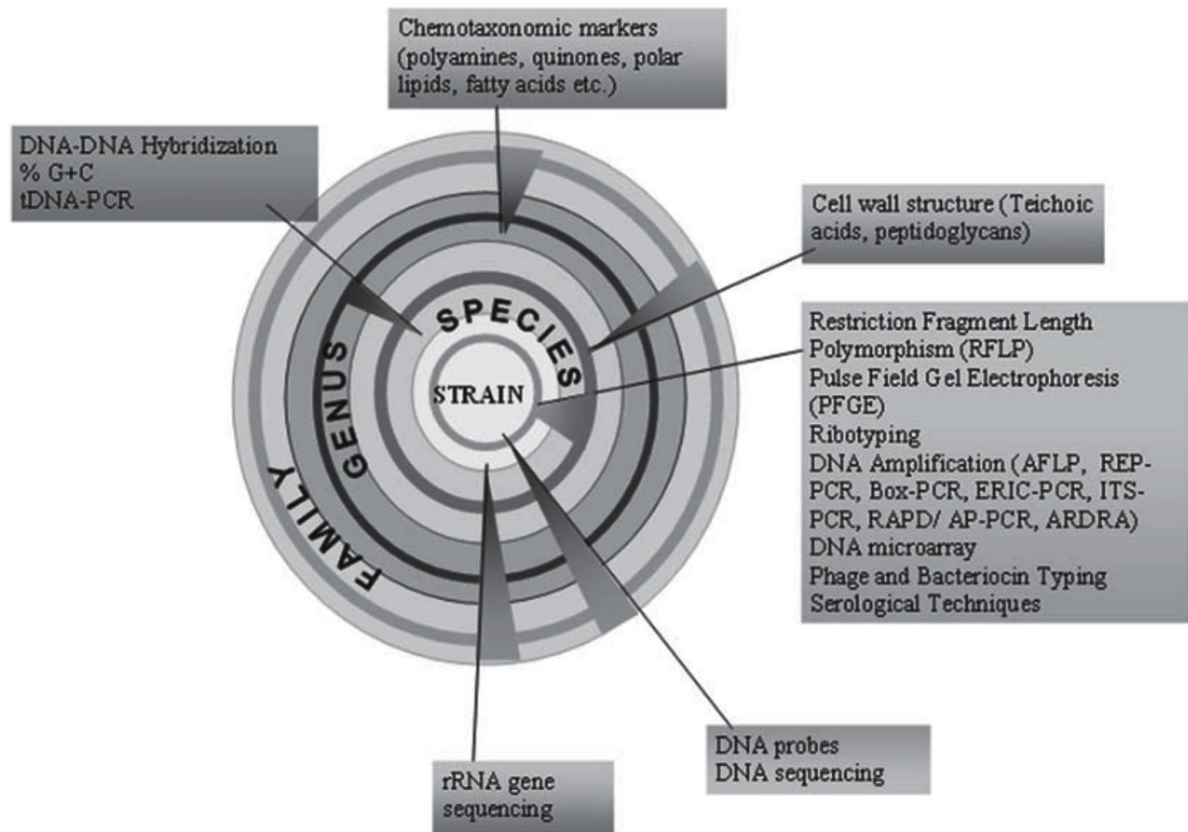


Figure 8. Diagrammatic representation of modern polyphasic approach in identifying *Actinobacteria* upto species level (Prem *et al.*, 2013)

CHAPTER II: MATERIALS & METHODS

CHAPTER II : Materials & methods

The actinobacterial genus *Kineococcus* was analyzed using chemotaxonomic methods (*Jaccard* and *Dice* indices) and molecular methods (16S rRNA and whole-genome sequencing). Fifteen species within the genus were studied, creating dendrograms to show their relationship to the reference species, *K. vitellinus*. The primary aim was to assess the feasibility of simpler taxonomic analyses as substitutes for intricate phylogenetic and phylogenomic studies in determining genetic proximity to a reference species.

2.1 Studied species

In this study, fifteen published species from the genus *Kineococcus* have been taken. The species are described briefly and given according to the publication date order:

2.1.1. *Kineococcus aurantiacus*

The strain was isolated from soil obtained from the Indore region of India. It is Gram-positive cocci. The *Actinobacteria* can be found in pairs, tetrads, and octads and occur in clusters. During any stage of growth or when the organism was cultured under various growth conditions, no rod-shaped or filamentous cells were observed. Had formed rough, round, convex, orange colonies on the majority of solid media. Nearly every cell exhibited active motility (Yokota *et al.*, 1993).

- Etymology: *aurantiacus* means orange-colored.

2.1.2. *Kineococcus radiotolerans*

The strain was isolated from a radioactive work area at the Savannah River Site in Aiken, South Carolina, USA. The colonies were orange and round with rough edges and coccus-shaped. The cells normally grew in clusters, but individual motile flagellated cells were also observed. The strain exhibited high levels of resistance to γ -radiation and desiccation (Phillips *et al.*, 2002).

- Etymology: *radiotolerans* referring to radiation-tolerating.

2.1.3. *Kineococcus gynurae*

The strain was isolated from the roots of a Thai medicinal plant in Bangkok, Thailand. The cells were strictly aerobic, motile, non-spore-forming, Gram-positive bacteria, and cocci-shaped. There were single, paired, and clustered cells. On GYE agar, the colonies were circular, convex, and dark orange in color (Duangmal *et al.*, 2008).

- Etymology: *gynurae* referring to gynura, isolated from the roots of a Thai medicinal plant called (*gynura pseudochina*).

2.1.4. *Kineococcus rhizosphaerae*

The strain was isolated from the rhizosphere soil of a cliff-associated plant *Peucedanum japonicum* Thunb. on Mara Island, Jeju, Republic of Korea. An orange-colored was observed with a phase-contrast optics. The cells were strictly aerobic, motile, Gram-positive staining, absence of hyphae and the formation of smooth and circular colonies (Lee, 2009).

- Etymology: *rhizosphaerae* referring to the site from which the type strain was isolated.

2.1.5. *Kineococcus xinjiangensis*

The strain was isolated from desert sand in Xinjiang Province, China. Gram-positive bacteria, strictly aerobic, motile, nonspore-forming, and cocci-shaped cells, absence of hyphae. The colonies are colored brown to orange and release a diffusible pigment that is brown in color (Liu *et al.*, 2009).

- Etymology: *xinjiangensis* referring to xinjiang, a province in north-west china, where the type strain was isolated.

2.1.6. *Kineococcus glutinatus*

The strain was isolated from soil taken in Dongchuan County, Yunnan Province, southwest China, from a dry, hot river valley. The cells had a coccoid shape, were aerobic, motile, Gram-positive bacteria, and lacked hyphae. Clusters of cells formed and tightly adhered to each other (Nie *et al.*, 2012).

- Etymology: *glutinatus* means glued together, agglutinated (i.e. cells occur in clusters and agglutinate strongly together).

2.1.7. *Kineococcus endophyticus*

The strain was isolated from a halophytic plant (*Limonium sinense*) that was collected from the east Chinese province of Jiangsu's coastal region of Nantong. The cells are motile, non-spore-forming, aerobic, Gram-positive staining, cocci-shaped, and lack hyphae. The colonies are circular, orange, and smooth (Bian *et al.*, 2012).

- Etymology: *endophyticus* means within a plant.

2.1.8. *Kineococcus gypseus*

The strain was isolated from alkaline sediment in Yuanjiang, Yunnan Province, southwest China. Gram-positive, spore-forming, motile, cocci-shaped, absence of hyphae, cells occur singly, in pairs, or in clusters. The tough, round colonies have a deep orange color (Li *et al.*, 2015).

- Etymology: *gypseus* referring to white with gypsum (covered by gypsum).

2.1.9. *Kineococcus mangrovi*

The strain was isolated from mangrove sediment in Thailand. Aerobic, coccus-shaped, motile, Gram-stain-positive, and nonspore-forming, absence of hyphae. The colonies are deep orange, rounded, and rough (Duangmal *et al.*, 2016).

- Etymology: *mangrovi* referring to the isolation of the type strain from a mangrove forest.

2.1.10. *Kineococcus terrestris*

The strain was isolated from alkaline sediment in Yuanjiang, China. The strain generated rough, orange colonies that were spherical and had Gram-positive, motile, non-spore-forming cells. There is no sign of hyphae. It appears as single cells, pairs, or clustered cells, measuring 1.0–1.3 μm in diameter, tightly adhered to one another. Flagella tufts were present in the motile cells (Xu *et al.*, 2017).

- Etymology: *terrestris* referring to the earth.

2.1.11. *Kineococcus aureolus*

The strain was isolated from alkaline sediment in Yuanjiang, China. The strain generated rough, orange colonies that were spherical and had Gram-positive, motile, non-spore-forming cells. There is no sign of hyphae. It appears as single cells, pairs, or clustered cells, measuring 1.0–1.3 μm in diameter, tightly adhered to one another. Flagella tufts were present in the motile cells (Xu *et al.*, 2017).

- Etymology: *aureoles* referring to the golden color of the colonies.

2.1.12. *Kineococcus rubinsiae*

The strain was isolated from a Jet Propulsion Laboratory spacecraft assembly cleanroom in Pasadena, CA, United States. The cells had a coccoid shape, the absence of hyphae, were motile, strictly aerobic, Gram-positive, and did not form spores. Round, rough-edged, orange-pigmented cells, clusters formed colonies, although individual motile cells were also seen (Mhatre *et al.*, 2021).

- Etymology: *rubinsiae* named in honor of a NASA astronaut (Kate Rubins) who is a molecular microbiologist and the first person to perform DNA sequencing in space.

2.1.13. *Kineococcus vitellinus*

The strain was originally isolated from biocrust samples in Almería, Spain, close to the Tabernas Desert. They have a cocci shape, motile, lack hyphae, Gram-positive, and do not form endospores. The cells can be found separated, in pairs, or in clusters. Colonies have a diameter of 1-3 mm and are round, rough, and pale orange in color (Molina-Menor *et al.*, 2021).

- Etymology: *vitellinus* referring to egg-yolk-coloured.

2.1.14. *Kineococcus indalonis*

The strain was originally isolated from biocrust samples in Almería, Spain, close to the Tabernas Desert. They have a cocci shape, are motile, lack hyphae, are Gram-positive, and do not form endospores. The cells can be found separated, in pairs, or in clusters. The irregularly shaped, rough, pale orange colonies have a diameter of only 1 mm. However, when the temperature drops below 20°C, the color turns dark greenish instead of orange (Molina-Menor *et al.*, 2021).

- Etymology: *indalonis* referring to a prehistoric symbol found in rock paintings in almería (spain), referring to the place where the microorganism was isolated.

2.1.15. *Kineococcus siccus*

The strain was originally isolated from biocrust samples in Almería, Spain, close to the Tabernas Desert. They are cocci-shaped, motile, lack hyphae, Gram-positive, and do not form endospores. The cells can be found separated, in pairs, or in clusters. Colonies are round, orange, with irregular margins and a variable size of 1-2 mm in diameter (Molina-Menor *et al.*, 2021).

- Etymology: *siccus* means dry.

2.2 Chemotaxonomic analysis

In this work, a chemotaxonomic analysis has been used to make a profile of all the *Kineococcus* species that have been reported since 1993 based on cellular components (sugar, amino acids, menaquinone, phospholipids, and fatty acids), and the data are shown very clearly in the results chapter below.

The scientific articles about the studied species that have been published have been carefully examined to provide data about these components. A binary value (1/0) indicates each component's presence or absence, respectively. The final data are displayed as tables, with the cellular components represented by the columns and the studied species represented by the rows.

2.2.1 Similarity calculation

When calculating the similarity, dissimilarity, or distance between the two sets, a variety of statistical techniques can be applied, and as many characteristics as possible could

be taken into account as well as the results. Two statistical coefficients are used in this study to determine how similar the species are to each other. The coefficients that are employed are the *Jaccard* and *Dice* coefficients.

1. Coefficient of *Jaccard*

The *Jaccard* coefficient is a correlation coefficient used to evaluate how similar two sample sets are. This metric is widely employed in chemical compound comparisons as a similarity measure (*Jaccard*, 1912).

The *Jaccard* coefficient, sometimes referred to as the *Jaccard* index or *Jaccard* similarity coefficient, is a metric used to express how similar two sets are to one another, and it is defined as the size of the intersection of the sets divided by the size of the union of the sets. In terms of math, it is written as:

$$J(A, B) = \frac{A \cap B}{A \cup B}$$

Where:

- (A) and (B) are two sets.
- (A) represents the number of elements in set A.
- (B) represents the number of elements in set B.
- (A∩B) represents the number of elements common to both sets (the intersection).
- (A∪B) represents the total number of unique elements in both sets (the union).

In our case it is written as:

$$J = \frac{a}{a+b+c}$$

Where:

- (a) represents the intersection in the matrix (1, 1).
- (b) represents the intersection in the matrix (0, 1).
- (c) represents the intersection in the matrix (1, 0).
- (d) represents the intersection in the matrix (0, 0).

	1	0
1	a (1,1)	b (0,1)
0	c (1,0)	d (0,0)

The range of the *Jaccard* coefficient is a number between 0 and 1, where 1 represents perfect similarity (the sets are identical) and 0 represents no similarity at all. Measuring the similarity between data sets is a widely used technique in many domains, such as data mining, information retrieval.

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.

$$J = \frac{1}{1+2+1} = 0.25 \rightarrow \text{The similarity between E1 and E2 is 25\%}.$$

2. Coefficient of *Dice*

The coefficient of *Dice*, also called the *Sørensen-Dice* similarity index, was independently created by *Dice* and *Sørensen* and is used to evaluate how similar two samples are to each other. This index was created with discrete data. It calculates the similarity by using three sets, a, b, and c, and the results variates between 0 and 1, where 1 represents perfect similarity (the two sets are identical) and 0 represents no similarity at all (*Dice*, 1945; *Sørensen*, 1948).

The *Dice* coefficient is similar to the *Jaccard* coefficient but is considered to be more appropriate for cases where the size of the intersection between the sets is small relative to the

size of the sets themselves. The definition of the *Dice* coefficient is twice the set intersection size divided by the total set sizes:

$$D(A, B) = 2 \cdot A \cap B / A + B$$

Where:

- (A) and (B) are two sets.
- $(A \cap B)$ represents the number of elements common to both sets (the intersection).
- (A) represents the number of elements in set A.
- (B) represents the number of elements in set B.

In our case it is written as:

$$D = \frac{2a}{2a + b + c}$$

Where:

- (a) represents the intersection in the matrix (1, 1).
- (b) represents the intersection in the matrix (0, 1).
- (c) represents the intersection in the matrix (1, 0).
- (d) represents the intersection in the matrix (0, 0).

	1	0
1	a (1,1)	b (0,1)
0	c (1,0)	d (0,0)

The range of the *Dice* coefficient is a number between 0 and 1, where 1 represents perfect similarity (the sets are identical) and 0 represents no similarity at all. Measuring the similarity between data sets is a widely used technique in many domains, such as data mining, information retrieval, and natural language processing.

3. Paleontological statistics 4.16c

PAST (Paleontological statistics) is a free software package designed for scientific statistical analysis in paleontology. It offers several statistical techniques designed especially for the analysis of paleontological data. With PAST, scientists can conduct a wide range of

analyses, including multivariate analysis, ecological and biogeographical analysis, and standard statistical tests that are frequently applied in paleontology. PAST version 4.16c (Figure 9) is the most recent version accessible.

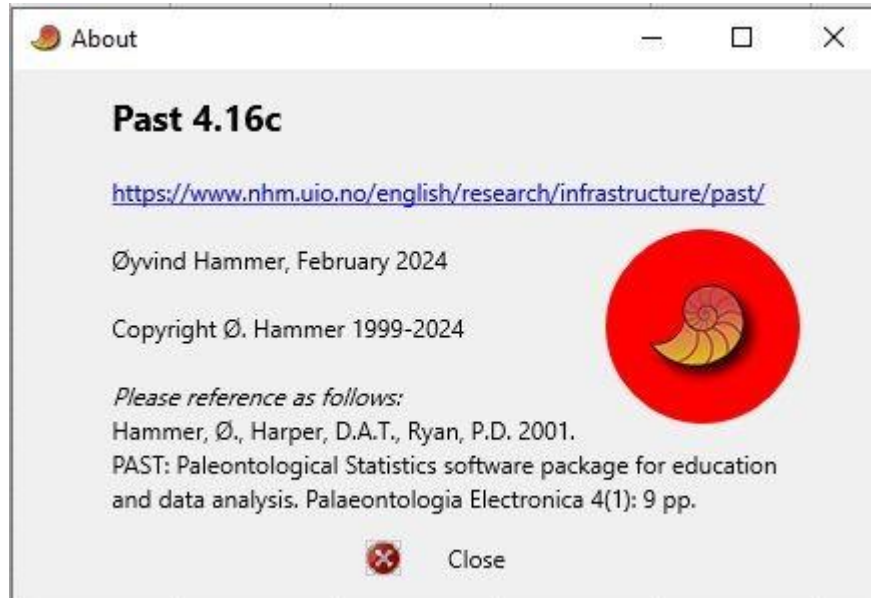


Figure 9. Paleontological statistics (PAST 4.16c).

The names of the species are arranged as rows in the chemotaxonomic data, while their characteristics are arranged as columns. The *Jaccard* coefficient and *Dice* coefficient are used to calculate the similarity matrices and determine the species' degree of similarity to *K. vitellinus*. The classical clustering method is used to build the dendrogram in the final phases.

2.3 Molecular analysis

In this section will have two studies which are phylogenetic study based on a single gene (16S rRNA gene) and phylogenomic study based on the whole genome (DNA).

2.3.1 Phylogenetic study (16S rRNA)

The 16S rRNA sequences of all the published and validated *Kineococcus* species are downloaded from a database website called EZBioCloud in FASTA format.

Based on the 16S rRNA gene sequences, the calculation of evolutionary distances to *K. vitellinus*, the last confirmed and published member in the genus *Kineococcus*. Using the neighbor-joining method in MEGA 11, a phylogenetic tree is created, and the evolutionary distances are compared to the similarity order that can be found on EZbiocloud.

2.3.2 EZBioCloud

EZBioCloud is a platform for online bioinformatics tools and databases with a primary focus on metagenomics and microbial genomics (Figure 10). It provides tools for sequence alignment, genome annotation, phylogenetic analysis, and comparative genomics, among other resources to help researchers analyze and interpret genomic data. Users can obtain extensive details regarding microbial taxonomy, diversity, and functional annotation by accessing the carefully selected microbial genome databases and reference datasets hosted by EZBioCloud. All things considered, EZBioCloud is a useful tool for researchers studying microbiology and microbial ecology.



Figure 10. EZBioCloud database

2.3.3 Molecular Evolutionary Genetics Analysis (MEGA 11)

Molecular Evolutionary Genetics Analysis Version 11 (Figure 11) is a widely used software tool for conducting molecular evolutionary analysis and constructing phylogenetic trees. MEGA provides a user-friendly interface and a comprehensive set of tools for analyzing DNA and protein sequence data. MEGA allows researchers to perform a variety of analyses, including:

1. **Sequence analyses:** Phylogeny inference, model selection, ancestral states, selection and tests, sequence alignment.
2. **Statistical methods:** Maximum likelihood, distance methods, ordinary least squares, maximum parsimony, composite likelihood, bayesian.
3. **Powerful visual tools:** Alignment/trace editor, tree explorer, data explorers, legend generator, gene duplication wizard, timetree wizard.

overall, mega is a versatile and powerful tool for molecular evolutionary analysis, widely used by researchers in fields such as evolutionary biology, genetics, and

bioinformatics. it continues to be updated to incorporate new features and improvements in analysis algorithms.



Figure 11. MEGA 11 tool

2.3.3.1 Neighbor-joining method (NJ)

Neighbor joining is a widely used method in phylogenetics for constructing evolutionary trees or phylogenetic trees. Phylogenetic trees depict the evolutionary relationships among a group of organisms or sequences, such as DNA or protein sequences.

The neighbor-joining method works by iteratively joining pairs of taxa (organisms or sequences) based on a measure of their pairwise evolutionary distances. At each step, it identifies the pair of taxa with the smallest distance between them and creates a new node to represent their common ancestor. This process continues until all taxa are joined into a single tree.

The key steps in the neighbor joining algorithm are:

1. **Calculate pairwise distances:** Compute a matrix of pairwise evolutionary distances between all pairs of taxa. These distances are typically estimated based on sequence alignment data or other relevant information.
2. **Compute the q-matrix:** From the pairwise distances, compute a matrix Q that represents the "quality" of joining each pair of taxa to form a new node in the tree.
3. **Identify minimum q-value:** Identify the pair of taxa with the smallest q-value, indicating the best candidate for calculation.
4. **Join the selected pair:** Create a new internal node in the tree to represent the common ancestor of the selected pair of taxa. Adjust the tree and update the distance matrix accordingly.
5. **Repeat:** Repeat steps 2-4 until all taxa have been joined into a single tree.

Neighbor joining is popular because it is relatively fast and efficient for constructing phylogenetic trees, especially with large datasets. However, it's worth noting that NJ doesn't always produce the most accurate trees, particularly when the evolutionary history involves complex processes such as gene duplication, horizontal gene transfer, or rapid evolution. In such cases, more sophisticated methods such as maximum Likelihood or Bayesian inference may be preferred.

Sequence alignments and phylogenetic tree construction are fundamental tasks in molecular evolutionary analysis, and MEGA provides tools to perform both of these tasks.

2.3.3.2 Sequence Alignment

MEGA allows users to align molecular sequences (such as DNA or protein sequences) using various algorithms like ClustalW, MUSCLE, or the built-in MEGA aligner Figure 12.

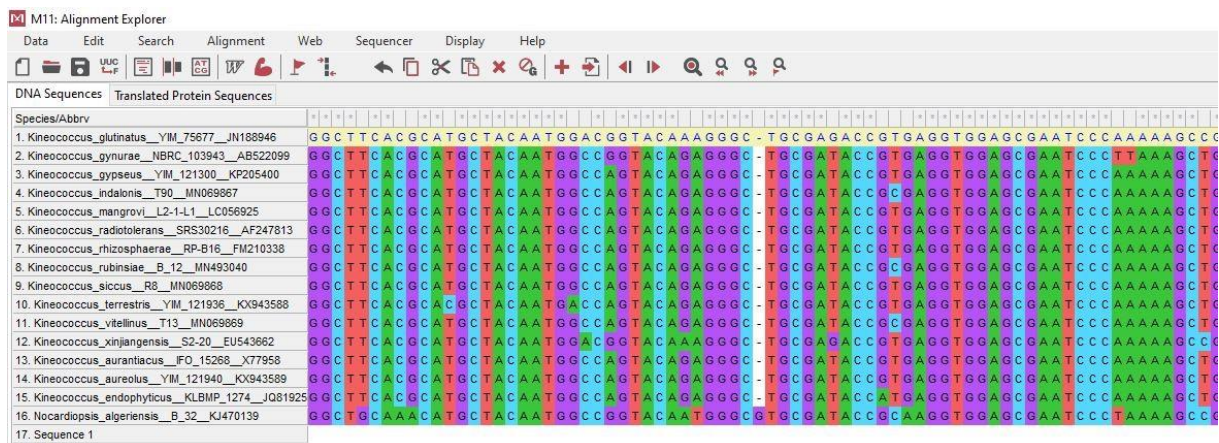


Figure 12. 16S rRNA gene sequences in MEGA 11 after alignment

2.3.3.3 Phylogenetic tree construction

MEGA supports various methods for tree construction, including neighbor-joining (NJ), maximum likelihood (ML), and maximum parsimony (MP).

2.3.4 Phylogenomic study (whole genome)

The DNA whole genome sequences of all the published and validated *Kineococcus* species are downloaded from a database website called NCBI in FASTA format.

2.3.4.1 National Center for Biotechnology Information (NCBI)

The National Center for Biotechnology Information (NCBI) database (Figure 13) is a comprehensive online resource provided by the National Institutes of Health (NIH) in the United States. It serves as a central repository for biological and biomedical information, offering a wide range of databases, tools, and resources for researchers, healthcare professionals, educators, and the general public.

NCBI hosts a vast collection of genomic databases, including GenBank, which stores annotated DNA sequences, as well as databases for other types of molecular data such as protein sequences, gene expression, and genetic variation.



Figure 13. National Center for Biotechnology Information (NCBI)

To build the hierarchical tree (phylogenetic tree) of the *Kineococcus* genus, we use the help of a website called TYGS, which offers a taxonomic classification of the whole genome.

2.3.4.2 TYGS (Type {Strain} Genome Server)

The TYGS (Type (Strain) Genome Server) website (Figure14) hosted by DSMZ (German Collection of Microorganisms and Cell Cultures) is an online platform dedicated to providing tools and resources for microbial genomics research. It specializes in the analysis and comparison of bacterial genomes, particularly focusing on type strains, which are the original strains from which a species was first described.

The TYGS website offers various features and services, including: genome analysis tools, comparative genomics, data repository and taxonomic classification.

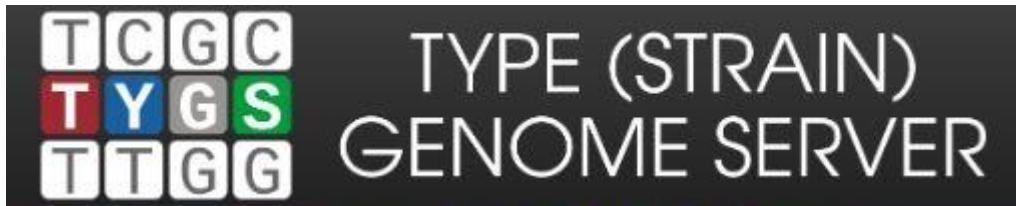


Figure 14. TYGS (Type (Strain) Genome Server)

2.3.5 ANI value

The ANI value (Average Nucleotide Identity) is used to classify and identify bacteria. It is commonly used to compare the genome sequences of two prokaryotes. The OrthoANIu algorithm is used by the ANI calculator in EZBioCloud.

ANI is often used to assess the relatedness or similarity between microbial strains or species. A higher ANI value indicates greater genetic similarity, suggesting that the two genomes likely belong to the same species or very closely related species. Conversely, a lower ANI value indicates less genetic similarity, suggesting greater evolutionary divergence or belonging to different species.

CHAPTER III: RESULTS & DISCUSSION

CHAPTER III : Results & discussion

3.1 Chemotaxonomy

Based on the published articles, the Tables are filled, and the chemotaxonomy results of the cellular components are shown below in a separate Table categorized by sugars, amino acids, menaquinones, polar lipids, and fatty acids.

Characteristic components are not taken into account quantitatively; they are simply recorded as present or absent (number 1 with a black-colored cell or number 0 with a white-colored cell).

3.1.1 Sugars content analysis

Galactose can be considered a biochemical marker sugar, as displayed by the information presented in Table 8, which reveals that it is found in all twelve species within the genus *Kineococcus*. This result validates the *Kineococcus*'s place inside the type A whole-cell sugar chemotype described by Labeda and Lechevalier (1989) research. Glucose, mannose, ribose, and arabinose are present variably (91.66%, 75.00%, 75.00%, and 66.66%, respectively).

K. aurantiacus, *K. rhizosphaerae*, and *K. glutinatus* are characterized by the presence of rhamnose. However, *K. rhizosphaerae*, is the only member of this genus that contains a trace amount of xylose in sugars, which could be considered as a distinctive feature.

The presence of other sugars, as shown in Table 8, is less or more characteristic.

Table 8. Whole-cell sugar content analysis of *Kineococcus*.

Species	Glu	Man	Gal	Rib	Ara	Xyl	Rham	Mad
<i>Kineococcus aurantiacus</i> Yokota <i>et al.</i> (1993)	+	+	+	+	+	-	+	-
<i>Kineococcus radiotolerans</i> Phillips <i>et al.</i> (2002)	+	+	+	+	+	-	-	-
<i>Kineococcus gynurae</i> Duangmal <i>et al.</i> (2008)	-	-	+	-	+	-	-	-
<i>Kineococcus rhizosphaerae</i> Lee (2009)	+	+	+	+	+	+	+	-
<i>Kineococcus xinjiangensis</i> Liu <i>et al.</i> (2009)	-	-	+	-	+	-	-	-
<i>Kineococcus glutinatus</i> Nie <i>et al.</i> (2012)	+	+	+	+	+	-	+	-
<i>Kineococcus endophyticus</i> Bian <i>et al.</i> (2012)	-	-	+	-	+	-	-	-
<i>Kineococcus gypseus</i> Li <i>et al.</i> (2015)	+	+	+	+	+	-	-	-
<i>Kineococcus mangrovi</i> Duangmal <i>et al.</i> (2016)	+	+	+	+	+	-	-	-
<i>Kineococcus terrestris</i> Xu <i>et al.</i> (2017)	+	+	+	+	+	-	-	-
<i>Kineococcus aureolus</i> Xu <i>et al.</i> (2017)	+	+	+	+	+	-	-	-
<i>Kineococcus rubinsiae</i> Mhatre <i>et al.</i> (2021)	+	+	+	-	-	-	-	-
<i>Kineococcus vitellinus</i> Molina-Menor <i>et al.</i> (2021)	ND	ND	ND	ND	ND	ND	ND	ND
<i>Kineococcus indalonis</i> Molina-Menor <i>et al.</i> (2021)	ND	ND	ND	ND	ND	ND	ND	ND
<i>Kineococcus siccus</i> Molina-Menor <i>et al.</i> (2021)	ND	ND	ND	ND	ND	ND	ND	ND
Frequency	9/15	9/15	12/15	8/15	11/15	1/15	3/15	0/15
	60.00%	60.00%	80.00%	53.33%	73.33%	6.66%	20.00%	0.00%

Black: positive; **white:** negative; **gray:** not determined; **Glu**, Glucose; **Man**, Mannose; **Gal**, Galactose; **Rib**, Ribose; **Ara**, Arabinose; **Xyl**, Xylose; **Mad**, Madurose.

3.1.2 Amino acids content analysis

All strains, with the exception of *K. radiotolerans*, *K. vitellinus*, *K. indalonis*, and *K. siccus*, which are unknown, consistently contained the diaminopimelic acid *meso*-DAP form, according to amino acid and peptidoglycan studies. Consequently, it is safe to say that the existence of *meso*-DAP serves as a chemical signature for the strains under study.

Glutamate and Alanine were present in *K. aurantiacus*, *K. glutinatus*, and *K. gypseus*. The *K. glutinatus* strain also contains significant amounts of threonine and asparagine.

Chemotaxonomic analysis was not performed on the last three strains that were published in 2021: *K. vitellinus*, *K. indalonis*, and *K. siccus*.

The presence of other amino acids and peptidoglycan, as shown in Table 9, is less or more characteristic.

Table 9. Amino acids and peptidoglycan content analysis of *Kineococcus*.

Species	Glu	Ala	Gly	GlcN	LL-DAP	meso-DAP	Asp	Thr	Mur	Mur NAc
<i>Kineococcus aurantiacus</i> Yokota <i>et al.</i> (1993)	+	+	-	-	-	+	-	-	-	-
<i>Kineococcus radiotolerans</i> Phillips <i>et al.</i> (2002)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Kineococcus gynurae</i> Duangmal <i>et al.</i> (2008)	-	-	-	-	-	+	-	-	-	-
<i>Kineococcus rhizosphaerae</i> Lee (2009)	-	-	-	-	-	+	-	-	-	-
<i>Kineococcus xinjiangensis</i> Liu <i>et al.</i> (2009)	-	-	-	-	-	+	-	-	-	-
<i>Kineococcus glutinatus</i> Nie <i>et al.</i> (2012)	+	+	-	-	-	+	+	+	-	-
<i>Kineococcus endophyticus</i> Bian <i>et al.</i> (2012)	-	-	-	-	-	+	-	-	-	-
<i>Kineococcus gypseus</i> Li <i>et al.</i> (2015)	+	+	-	-	-	+	-	-	-	-
<i>Kineococcus mangrovi</i> Duangmal <i>et al.</i> (2016)	-	-	-	-	-	+	-	-	-	-
<i>Kineococcus terrestris</i> Xu <i>et al.</i> (2017)	-	-	-	-	-	+	-	-	-	-
<i>Kineococcus aureolus</i> Xu <i>et al.</i> (2017)	-	-	-	-	-	+	-	-	-	-
<i>Kineococcus rubinsiae</i> Mhatre <i>et al.</i> (2021)	-	-	-	-	-	+	-	-	-	-
<i>Kineococcus vitellinus</i> Molina-Menor <i>et al.</i> (2021)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Kineococcus indalonis</i> Molina-Menor <i>et al.</i> (2021)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Kineococcus siccus</i> Molina-Menor <i>et al.</i> (2021)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Frequency	3/15	3/15	0/15	0/15	0/15	11/15	1/15	1/15	0/15	0/15
	20.00%	20.00%	0.00%	0.00%	0.00%	73.33%	6.66%	6.66%	0.00%	0.00%

Black: positive; **white:** negative; **gray:** not determined; **Glu:** Glutamate; **Ala:** Alanine; **Gly:** Glycine; **GlcN:** Glucosamine; **LL-DAP:** LL-Diaminopimelic acid;

ASP: asparagine; **meso-DAP:** meso-Diaminopimelic acid; **Mur:** Muramic acid; **MurNAc:** N-acetylmuramic acid.

3.1.3 Menaquinones content analysis

The menaquinones study profile made it abundantly evident that MK-9 (H₂) is the most characteristic menaquinone in the *Kineococcus* genus, with the exception of four strains due to a lack of data in their artefals.

The results are shown in Table 10 below.

Table 10. Menaquinones content analysis of *Kineococcus*.

Species	MK-7(H ₂)	MK-7(H ₄)	MK-8(H ₂)	MK-8(H ₄)	MK-8(H ₆)	MK-9(H ₂)	MK-9(H ₄)	MK-9(H ₆)	MK-10(H ₄)
<i>Kineococcus aurantiacus</i> Yokota <i>et al.</i> (1993)	-	-	-	-	-	+	-	-	-
<i>Kineococcus radiotolerans</i> Phillips <i>et al.</i> (2002)	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Kineococcus gynurae</i> Duangmal <i>et al.</i> (2008)	-	-	-	-	-	+	-	-	-
<i>Kineococcus rhizosphaerae</i> Lee (2009)	-	-	-	-	-	+	-	-	-
<i>Kineococcus xinjiangensis</i> Liu <i>et al.</i> (2009)	-	-	-	-	-	+	-	-	-
<i>Kineococcus glutinatus</i> Nie <i>et al.</i> (2012)	-	-	-	-	-	+	-	-	-
<i>Kineococcus endophyticus</i> Bian <i>et al.</i> (2012)	-	-	-	-	-	+	-	-	-
<i>Kineococcus gypseus</i> Li <i>et al.</i> (2015)	-	-	-	-	-	+	-	-	-
<i>Kineococcus mangrovi</i> Duangmal <i>et al.</i> (2016)	-	-	-	-	-	+	-	-	-
<i>Kineococcus terrestris</i> Xu <i>et al.</i> (2017)	-	-	-	-	-	+	-	-	-
<i>Kineococcus aureolus</i> Xu <i>et al.</i> (2017)	-	-	-	-	-	+	-	-	-
<i>Kineococcus rubinsiae</i> Mhatre <i>et al.</i> (2021)	-	-	-	-	-	+	-	-	-

Black: positive; **white:** negative; **gray:** not determined.

Table 10 Continued- Menaquinones content analysis of *Kineococcus*.

Species	MK-7(H ₂)	MK-7(H ₄)	MK-8(H ₂)	MK-8(H ₄)	MK-8(H ₆)	MK-9(H ₂)	MK-9(H ₄)	MK-9(H ₆)	MK-10(H ₄)
<i>Kineococcus vitellinus</i> Molina-Menor <i>et al.</i> (2021)	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Kineococcus indalonis</i> Molina-Menor <i>et al.</i> (2021)	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Kineococcus siccus</i> Molina-Menor <i>et al.</i> (2021)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Frequency	0/15	0/15	0/15	0/15	0/15	11/15	0/15	0/15	0/15
	0.0%	0.0%	0.0%	0.0%	0.0%	73.33%	0.0%	0.0%	0.0%

Black: positive; **white:** negative; **gray:** not determined.

3.1.4 Polar lipids analysis

The results in Table 11 showed that diphosphatidylglycerol and phosphatidylglycerol with a frequency of (13/15) are the most represented phospholipids, followed by phosphatidylinositol with a frequency of (08/15), unknown phosphoglycolipids with a frequency of (05/15), phosphatidylinositol mannosides with a frequency of (05/15), and unknown phospholipids with a frequency of (05/15), followed by a trace of the other phospholipids in some strains. The results are shown in Table 11 below.

Table 11. Phospholipid and polar lipids content analysis of *Kineococcus*.

Species	PE	DPG	PI	PG	HPE	PC	PME	LPE
<i>Kineococcus aurantiacus</i> Yokota <i>et al.</i> (1993)	-	+	-	+	-	-	-	-
<i>Kineococcus radiotolerans</i> Phillips <i>et al.</i> (2002)	-	+	+	+	-	-	-	-
<i>Kineococcus gynurae</i> Duangmal <i>et al.</i> (2008)	-	-	-	-	-	-	-	-
<i>Kineococcus rhizosphaerae</i> Lee (2009)	-	+	+	+	-	-	-	-
<i>Kineococcus xinjiangensis</i> Liu <i>et al.</i> (2009)	-	-	-	-	-	-	-	-
<i>Kineococcus glutinatus</i> Nie <i>et al.</i> (2012)	+	+	+	+	-	-	-	-
<i>Kineococcus endophyticus</i> Bian <i>et al.</i> (2012)	-	+	+	+	-	-	-	-
<i>Kineococcus gypseus</i> Li <i>et al.</i> (2015)	-	+	+	+	-	-	-	-
<i>Kineococcus mangrovi</i> Duangmal <i>et al.</i> (2016)	-	+	-	+	-	-	-	-
<i>Kineococcus terrestris</i> Xu <i>et al.</i> (2017)	-	+	+	+	-	-	-	-
<i>Kineococcus aureolus</i> Xu <i>et al.</i> (2017)	-	+	+	+	-	-	-	-
<i>Kineococcus rubinsiae</i> Mhatre <i>et al.</i> (2021)	-	+	+	+	-	-	-	-
<i>Kineococcus vitellinus</i> Molina-Menor <i>et al.</i> (2021)	-	+	-	+	-	-	-	-
<i>Kineococcus indalonis</i> Molina-Menor <i>et al.</i> (2021)	-	+	-	+	-	-	-	-
<i>Kineococcus siccus</i> Molina-Menor <i>et al.</i> (2021)	-	+	-	+	-	-	-	-
Frequency	1/15	13/15	8/15	13/15	0/15	0/15	0/15	0/15
	6.66%	86.66%	53.33%	86.66%	0.00%	0.00%	0.00%	0.00%

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

Table 11 Continued- phospholipids and polar lipids content analysis of *Kineococcus*.

Species	PGL	PIM	PL	GL	L	PLs	NPG	AP
<i>Kineococcus aurantiacus</i> Yokota <i>et al.</i> (1993)	+	-	+	+	-	-	-	-
<i>Kineococcus radiotolerans</i> Phillips <i>et al.</i> (2002)	-	-	+	-	-	+	-	-
<i>Kineococcus gynurae</i> Duangmal <i>et al.</i> (2008)	-	-	-	-	-	-	-	-
<i>Kineococcus rhizosphaerae</i> Lee (2009)	-	-	+	-	-	+	-	-
<i>Kineococcus xinjiangensis</i> Liu <i>et al.</i> (2009)	-	-	-	-	-	-	-	-
<i>Kineococcus glutinatus</i> Nie <i>et al.</i> (2012)	-	+	+	-	-	-	-	-
<i>Kineococcus endophyticus</i> Bian <i>et al.</i> (2012)	-	+	+	+	+	-	-	-
<i>Kineococcus gypseus</i> Li <i>et al.</i> (2015)	-	+	+	-	-	-	-	-
<i>Kineococcus mangrovi</i> Duangmal <i>et al.</i> (2016)	+	-	-	-	-	-	-	-
<i>Kineococcus terrestris</i> Xu <i>et al.</i> (2017)	+	+	-	-	-	+	-	-
<i>Kineococcus aureolus</i> Xu <i>et al.</i> (2017)	+	+	-	-	-	+	-	-
<i>Kineococcus rubinsiae</i> Mhatre <i>et al.</i> (2021)	-	-	-	-	-	+	-	-
<i>Kineococcus vitellinus</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	-	-	-	-
<i>Kineococcus indalonis</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	-	-	-	-
<i>Kineococcus siccus</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	-	-	-	-
Frequency	4/15	5/15	6/15	2/15	1/15	5/15	0/15	0/15
	26.66%	33.33%	40%	13.33%	6.66%	33.33%	0.00%	0.00%

Black, positive; **white**, negative; **PGL**, unknown phosphoglycolipid; **PIM**, phosphatidylinositol mannosides; **PL**, unknown phospholipids; **GL**, unknown glycolipid; **NPG**, ninhydrin-positive phosphoglycolipid; **AP**, aminophosphate.

3.1.5 Fatty acids analysis

Anteiso C_{15:0} can be considered a biochemical marker fatty acid, as displayed by the information presented in Tables 12,13,14,15,16 and 17 which reveals that it is found in all fifteen species within the genus *Kineococcus*.

isoC_{14:0}, C_{14:0}, and C_{16:0} are present variably (86.66%, 80%, and 73.33%, respectively).

Table 12. Fatty acids content analysis of *Kineococcus* (a).

Species	C _{12:0}	C _{13:0}	C _{14:0}	C _{15:0}	C _{16:0}	C _{18:0}	C _{19:0}	C _{13:1}
<i>Kineococcus aurantiacus</i> Yokota <i>et al.</i> (1993)	+	+	+	-	+	+	-	-
<i>Kineococcus radiotolerans</i> Phillips <i>et al.</i> (2002)	-	-	-	-	+	-	-	-
<i>Kineococcus gynurae</i> Duangmal <i>et al.</i> (2008)	-	-	+	-	-	-	-	-
<i>Kineococcus rhizosphaerae</i> Lee (2009)	-	-	+	-	+	+	-	-
<i>Kineococcus xinjiangensis</i> Liu <i>et al.</i> (2009)	-	-	-	-	-	-	-	-
<i>Kineococcus glutinatus</i> Nie <i>et al.</i> (2012)	+	+	+	-	+	-	-	-
<i>Kineococcus endophyticus</i> Bian <i>et al.</i> (2012)	-	-	+	-	-	-	-	-
<i>Kineococcus gypseus</i> Li <i>et al.</i> (2015)	+	+	+	-	+	-	-	-
<i>Kineococcus mangrovi</i> Duangmal <i>et al.</i> (2016)	-	-	+	-	+	-	-	+
<i>Kineococcus terrestris</i> Xu <i>et al.</i> (2017)	-	-	+	-	+	-	-	-
<i>Kineococcus aureolus</i> Xu <i>et al.</i> (2017)	-	-	-	-	+	+	-	-
<i>Kineococcus rubinsiae</i> Mhatre <i>et al.</i> (2021)	+	-	+	-	+	+	+	-
<i>Kineococcus vitellinus</i> Molina-Menor <i>et al.</i> (2021)	-	-	+	-	-	-	-	-
<i>Kineococcus indalonis</i> Molina-Menor <i>et al.</i> (2021)	-	-	+	+	+	-	-	-
<i>Kineococcus siccus</i> Molina-Menor <i>et al.</i> (2021)	-	-	+	-	+	+	-	-
Frequency	4/15	3/15	12/15	1/15	11/15	5/15	1/15	1/15
	26.66%	20.00%	80.00%	6.66%	73.33%	33.33%	6.66%	6.66%

Black, positive; **white**, negative.

Table 13. Fatty acids content analysis of *Kineococcus* (b).

Species	C _{15:1}	C _{13:0} 2OH	C _{14:0} 2OH	C _{15:0} 2OH	C _{17:0} 2OH	C _{13:0} 3OH	C _{14:0} 3OH	C _{16:0} 3OH
<i>Kineococcus aurantiacus</i> Yokota <i>et al.</i> (1993)	-	+	+	+	+	-	-	-
<i>Kineococcus radiotolerans</i> Phillips <i>et al.</i> (2002)	-	-	-	-	-	-	-	-
<i>Kineococcus gynurae</i> Duangmal <i>et al.</i> (2008)	-	-	+	-	+	-	-	-
<i>Kineococcus rhizosphaerae</i> Lee (2009)	-	-	+	-	+	-	-	-
<i>Kineococcus xinjiangensis</i> Liu <i>et al.</i> (2009)	-	-	-	-	-	-	-	-
<i>Kineococcus glutinatus</i> Nie <i>et al.</i> (2012)	-	-	-	-	+	-	-	-
<i>Kineococcus endophyticus</i> Bian <i>et al.</i> (2012)	-	-	+	-	+	-	-	-
<i>Kineococcus gypseus</i> Li <i>et al.</i> (2015)	-	+	-	+	+	-	-	+
<i>Kineococcus mangrovi</i> Duangmal <i>et al.</i> (2016)	+	-	+	-	+	+	+	-
<i>Kineococcus terrestris</i> Xu <i>et al.</i> (2017)	-	-	-	-	+	-	-	-
<i>Kineococcus aureolus</i> Xu <i>et al.</i> (2017)	-	-	-	-	+	-	-	-
<i>Kineococcus rubinsiae</i> Mhatre <i>et al.</i> (2021)	-	-	+	-	+	-	-	+
<i>Kineococcus vitellinus</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	-	-	-	-
<i>Kineococcus indalonis</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	-	-	-	-
<i>Kineococcus siccus</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	-	-	-	-
Frequency	1/15	2/15	6/15	2/15	10/15	1/15	1/15	2/15
	6.66%	13.33%	40.00%	13.33%	66.66%	6.66%	6.66%	13.33%

Black, positive; **white**, negative.

Table 14. Fatty acids content analysis of *Kineococcus* (c).

Species	C _{17:0} 3OH	C _{17:1} 3OH	C _{18:0} 3OH	C _{16:0} N alcohol	C _{17:1} w7c	C _{18:1} w9c	C _{20:2} w6,9c	C _{20:4} w6,9,12,15c
<i>Kineococcus aurantiacus</i> Yokota <i>et al.</i> (1993)	+	-	+	+	+	+	-	-
<i>Kineococcus radiotolerans</i> Phillips <i>et al.</i> (2002)	+	-	-	+	+	-	-	-
<i>Kineococcus gynurae</i> Duangmal <i>et al.</i> (2008)	-	+	-	-	-	-	-	-
<i>Kineococcus rhizosphaerae</i> Lee (2009)	+	-	+	+	-	-	-	-
<i>Kineococcus xinjiangensis</i> Liu <i>et al.</i> (2009)	-	-	-	-	-	-	-	-
<i>Kineococcus glutinatus</i> Nie <i>et al.</i> (2012)	+	-	-	-	-	+	-	-
<i>Kineococcus endophyticus</i> Bian <i>et al.</i> (2012)	+	-	-	-	-	-	-	-
<i>Kineococcus gypseus</i> Li <i>et al.</i> (2015)	-	-	-	-	-	-	-	-
<i>Kineococcus mangrovi</i> Duangmal <i>et al.</i> (2016)	+	-	-	-	+	-	-	-
<i>Kineococcus terrestris</i> Xu <i>et al.</i> (2017)	+	-	-	+	-	-	-	-
<i>Kineococcus aureolus</i> Xu <i>et al.</i> (2017)	+	-	-	+	-	-	-	-
<i>Kineococcus rubinsiae</i> Mhatre <i>et al.</i> (2021)	-	-	-	+	-	+	+	+
<i>Kineococcus vitellinus</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	-	-	-	-
<i>Kineococcus indalonis</i> Molina-Menor <i>et al.</i> (2021)	+	-	-	-	-	-	-	-
<i>Kineococcus siccus</i> Molina-Menor <i>et al.</i> (2021)	+	-	-	-	-	-	-	-
Frequency	10/15	1/15	2/15	6/15	3/15	3/15	1/15	1/15
	66.66%	6.66%	13.33%	40.00%	20.00%	20.00%	6.66%	6.66%

Black, positive; **white**, negative.

Table 15. Fatty acids content analysis of *Kineococcus* (d).

Species	iso-C _{13:0}	iso-C _{13:0} 3OH	Iso-C _{14:0}	iso-C _{14:0} 3OH	Iso-C _{15:0}	iso-C _{15:1}	Iso-C _{16:0}	Iso-C _{16:1}
<i>Kineococcus aurantiacus</i> Yokota <i>et al.</i> (1993)	-	+	+	-	+	-	+	-
<i>Kineococcus radiotolerans</i> Phillips <i>et al.</i> (2002)	-	-	+	-	-	-	+	-
<i>Kineococcus gynurae</i> Duangmal <i>et al.</i> (2008)	-	-	+	-	-	-	-	-
<i>Kineococcus rhizosphaerae</i> Lee (2009)	-	-	+	-	+	-	+	-
<i>Kineococcus xinjiangensis</i> Liu <i>et al.</i> (2009)	-	-	-	-	-	-	-	-
<i>Kineococcus glutinatus</i> Nie <i>et al.</i> (2012)	+	-	+	-	+	-	+	-
<i>Kineococcus endophyticus</i> Bian <i>et al.</i> (2012)	-	-	+	-	+	-	-	-
<i>Kineococcus gypseus</i> Li <i>et al.</i> (2015)	-	-	+	+	+	+	-	-
<i>Kineococcus mangrovi</i> Duangmal <i>et al.</i> (2016)	-	+	+	+	+	-	+	+
<i>Kineococcus terrestris</i> Xu <i>et al.</i> (2017)	-	-	+	-	-	-	-	-
<i>Kineococcus aureolus</i> Xu <i>et al.</i> (2017)	-	-	+	-	-	-	-	-
<i>Kineococcus rubinsiae</i> Mhatre <i>et al.</i> (2021)	-	-	+	-	-	-	-	-
<i>Kineococcus vitellinus</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	+	-	-	-
<i>Kineococcus indalonis</i> Molina-Menor <i>et al.</i> (2021)	-	-	+	-	+	-	-	-
<i>Kineococcus siccus</i> Molina-Menor <i>et al.</i> (2021)	-	-	+	-	-	-	-	-
Frequency	1/15	2/15	13/15	2/15	8/15	1/15	5/15	1/15
	6.66%	13.33%	86.66%	13.33%	53.33%	6.66%	33.33%	6.66%

Black, positive; **white**, negative.

Table 16. Fatty acids content analysis of *Kineococcus* (e).

Species	iso-C _{16:1} H	iso-C _{16:0} 2OH	iso-C _{17:0}	iso-C _{17:1} w5c	iso-C _{17:1} w9c	iso-C _{17:0} 3OH	anteiso C _{13:0}	anteiso-C _{15:0}
<i>Kineococcus aurantiacus</i> Yokota <i>et al.</i> (1993)	-	-	-	-	-	-	-	+
<i>Kineococcus radiotolerans</i> Phillips <i>et al.</i> (2002)	-	-	-	-	-	-	-	+
<i>Kineococcus gynurae</i> Duangmal <i>et al.</i> (2008)	-	-	-	-	-	-	-	+
<i>Kineococcus rhizosphaerae</i> Lee (2009)	-	-	-	-	-	-	-	+
<i>Kineococcus xinjiangensis</i> Liu <i>et al.</i> (2009)	-	-	-	-	-	-	-	+
<i>Kineococcus glutinatus</i> Nie <i>et al.</i> (2012)	-	-	+	-	-	+	+	+
<i>Kineococcus endophyticus</i> Bian <i>et al.</i> (2012)	-	-	-	-	-	-	-	+
<i>Kineococcus gypseus</i> Li <i>et al.</i> (2015)	+	-	-	-	-	-	-	+
<i>Kineococcus mangrovi</i> Duangmal <i>et al.</i> (2016)	-	-	-	+	-	-	-	+
<i>Kineococcus terrestris</i> Xu <i>et al.</i> (2017)	-	-	-	-	-	-	-	+
<i>Kineococcus aureolus</i> Xu <i>et al.</i> (2017)	-	-	-	-	-	-	-	+
<i>Kineococcus rubinsiae</i> Mhatre <i>et al.</i> (2021)	-	-	-	-	-	-	-	+
<i>Kineococcus vitellinus</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	-	-	-	+
<i>Kineococcus indalonis</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	-	-	-	+
<i>Kineococcus siccus</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	-	-	-	+
Frequency	1/15	0/15	1/15	1/15	0/15	1/15	1/15	15/15
	6.66%	0.00%	6.66%	6.66%	0.00%	6.66%	6.66%	100.00%

Black, positive; **white**, negative.

Table 17. Fatty acids content analysis of *Kineococcus* (f).

Species	anteiso-C _{15:1}	anteiso-C _{16:0}	anteiso-C _{17:0}	anteiso-C _{17:1}	anteiso-C _{17:0} 2OH	C _{18:3} w6c (6,9,12)	C _{20:4} w6,9,12,15c	C _{19:0} 10- methyl	10 Methyl 1 C _{16:0}
<i>Kineococcus aurantiacus</i> Yokota <i>et al.</i> (1993)	+	-	+	-	-	+	-	-	-
<i>Kineococcus radiotolerans</i> Phillips <i>et al.</i> (2002)	+	-	-	-	-	-	-	-	-
<i>Kineococcus gynurae</i> Duangmal <i>et al.</i> (2008)	-	-	-	-	-	-	-	-	-
<i>Kineococcus rhizosphaerae</i> Lee (2009)	+	-	-	-	-	-	-	-	-
<i>Kineococcus xinjiangensis</i> Liu <i>et al.</i> (2009)	-	-	-	-	-	-	-	-	-
<i>Kineococcus glutinatus</i> Nie <i>et al.</i> (2012)	-	-	+	-	-	+	-	-	-
<i>Kineococcus endophyticus</i> Bian <i>et al.</i> (2012)	+	-	-	-	-	-	-	-	-
<i>Kineococcus gypseus</i> Li <i>et al.</i> (2015)	+	-	-	-	-	+	+	-	-
<i>Kineococcus mangrovi</i> Duangmal <i>et al.</i> (2016)	-	-	-	-	-	-	-	-	-
<i>Kineococcus terrestris</i> Xu <i>et al.</i> (2017)	+	-	-	-	-	-	-	-	-
<i>Kineococcus aureolus</i> Xu <i>et al.</i> (2017)	+	-	-	-	-	+	-	+	-
<i>Kineococcus rubinsiae</i> Mhatre <i>et al.</i> (2021)	+	-	-	-	-	-	-	-	-
<i>Kineococcus vitellinus</i> Molina-Menor <i>et al.</i> (2021)	+	-	-	-	-	-	-	-	-
<i>Kineococcus indalonis</i> Molina-Menor <i>et al.</i> (2021)	+	-	-	-	-	-	-	-	-
<i>Kineococcus siccus</i> Molina-Menor <i>et al.</i> (2021)	-	-	-	-	-	-	-	-	-
Frequency	10/15	0/15	2/15	0/15	0/15	4/15	1/15	1/15	0/15
	66.66%	0.00%	13.33%	0.00%	0.00%	26.66%	6.66%	6.66%	0.00%

Black, positive; **white**, negative.

3.2 Similarity based on *Jaccard* and *Dice* coefficients

According to *Jaccard* and *Dice* coefficients, the chemotaxonomic similarity results have produced varying similarity orders and degrees.

The obtained results present the percentage of similarity compared to the first reference species (*K. rubinsiae*). The species are classified in decreasing order according to the *Jaccard* coefficient (Table 18), which is as follows:

K. rubinsiae (RU) > *K. aureoles* (AURE) = *K. terrestris* (TE) > *K. rhizosphaerae* (RH) > *K. mangrovi* (MA) > *K. gypseus* (GYP) > *K. endophyticus* (EN) > *K. glutinatus* (GL) > *K. aurantiacus* (AURA) > *K. gynurae* (GYN) = *K. xinjiangensis* (XI).

The strains *K. radiotolerans* (RA), *K. vitellinus* (VI), *K. indalonis* (IN), and *K. siccus* (SI) won't be mentioned in Table 18 due to a lack of data.

Table 18 Calculation of similarity by coefficient of *Jaccard* for sugars, amino acids, menaquinones and phospholipids.

Species	a (1;1)	b (0;1)	c (1;0)	d (0;0)	$J = \frac{a}{a+b+c}$	Persentege
<i>K. rubinsiae</i> / <i>K. rubinsiae</i>	9	0	0	31	1	100%
<i>K. rubinsiae</i> / <i>K. aureolus</i>	9	4	0	27	0.6923	69.23%
<i>K. rubinsiae</i> / <i>K. terrestris</i>	9	4	0	27	0.6923	69.23%
<i>K. rubinsiae</i> / <i>K. mangrovi</i>	7	3	2	28	0.5833	58.33%
<i>K. rubinsiae</i> / <i>K. gypseus</i>	8	6	1	25	0.5333	53.33%
<i>K. rubinsiae</i> / <i>K. endophyticus</i>	6	5	3	26	0.4285	42.85%
<i>K. rubinsiae</i> / <i>K. glutinatus</i>	8	10	1	21	0.4210	42.10%
<i>K. rubinsiae</i> / <i>K. xinjiangensis</i>	3	1	6	30	0.3	30%
<i>K. rubinsiae</i> / <i>K. rhizosphaerae</i>	9	5	0	26	0.6428	64.28%
<i>K. rubinsiae</i> / <i>K. gynurae</i>	3	1	6	30	0.3	30%
<i>K. rubinsiae</i> / <i>K. aurantiacus</i>	7	8	2	23	0.4117	41.17%
<i>K. rubinsiae</i> / <i>K. radiotolerans</i>	ND	ND	ND	ND	ND	ND
<i>K. rubinsiae</i> / <i>K. vitellinus</i>	ND	ND	ND	ND	ND	ND
<i>K. rubinsiae</i> / <i>K. indalonis</i>	ND	ND	ND	ND	ND	ND
<i>K. rubinsiae</i> / <i>K. siccus</i>	ND	ND	ND	ND	ND	ND

ND, not determined

The obtained results present the percentage of similarity compared to the reference species (*K. vitellinus*). The species are classified in decreasing order according to the *Jaccard* coefficient (Table 19), which is as follows:

K. vitellinus (VI) > *K. indalonis* (IN) = *K. endophyticus* (EN) > *K. terrestris* (TE) > *K. rhizosphaerae* (RH) > *K. siccus* (SI) = *K. xinjiangensis* (XI) = *K. gynurae* (GYN) > *K. gypseus* (GYP) > *K. radiotolerans* (RA) > *K. rubinsiae* (RU) > *K. aurantiacus* (AURA) > *K. glutinatus* (GL) = *K. aureoles* (AU) > *K. mangrovi* (MA).

Table 19. Calculation of similarity by coefficient of *Jaccard* fatty acids.

Species	a (1;1)	b (0;1)	c (1;0)	d (0;0)	$J = \frac{a}{a+b+c}$	percentege
<i>K. vitellinus</i> / <i>K. vitellinus</i>	4	0	0	45	1	100%
<i>K. vitellinus</i> / <i>K. indalonis</i>	4	4	0	41	0.5	50%
<i>K. vitellinus</i> / <i>K. siccus</i>	2	4	2	41	0.25	25%
<i>K. vitellinus</i> / <i>K. rubinsiae</i>	3	12	1	33	0.1875	18.75%
<i>K. vitellinus</i> / <i>K. aureolus</i>	2	8	2	37	0.1666	16.66%
<i>K. vitellinus</i> / <i>K. terrestris</i>	3	5	1	40	0.3333	33.33%
<i>K. vitellinus</i> / <i>K. mangrovi</i>	3	15	1	30	0.1578	15.78%
<i>K. vitellinus</i> / <i>K. gypseus</i>	4	13	0	32	0.2352	23.52%
<i>K. vitellinus</i> / <i>K. endophyticus</i>	4	4	0	41	0.5	50%
<i>K. vitellinus</i> / <i>K. glutinatus</i>	3	14	1	31	0.1666	16.66%
<i>K. vitellinus</i> / <i>K. xinjiangensis</i>	1	0	3	45	0.25	25%
<i>K. vitellinus</i> / <i>K. rhizosphaerae</i>	4	9	0	36	0.3076	30.76%
<i>K. vitellinus</i> / <i>K. gynurae</i>	2	4	2	41	0.25	25%
<i>K. vitellinus</i> / <i>K. radiotolerans</i>	2	6	2	39	0.2	20%
<i>K. vitellinus</i> / <i>K. aurantiacus</i>	4	18	0	27	0.1818	18.18%

The results obtained present the percentage of similarity compared to the first reference species (*K. rubinsiae*). The species are classified in decreasing order according to the *Dice* coefficient (Table 20), which is as follows:

Table 20. Calculation of similarity by coefficient of *Dice* for sugars, amino acids, menaquinones and phospholipids.

Species	a (1;1)	b (0;1)	c (1;0)	d (0;0)	$D = \frac{2a}{2a+b+c}$	percentege
<i>K. rubinsiae</i> / <i>K. rubinsiae</i>	9	0	0	31	1	100%
<i>K. rubinsiae</i> / <i>K. aureolus</i>	9	4	0	27	0.8181	81.81%
<i>K. rubinsiae</i> / <i>K. terrestris</i>	9	4	0	27	0.8181	81.81%
<i>K. rubinsiae</i> / <i>K. mangrovi</i>	7	3	2	28	0.7368	73.68%
<i>K. rubinsiae</i> / <i>K. gypseus</i>	8	6	1	25	0.6956	69.56%
<i>K. rubinsiae</i> / <i>K. endophyticus</i>	6	5	3	26	0.6	60%
<i>K. rubinsiae</i> / <i>K. glutinatus</i>	8	10	1	21	0.5925	59.25%
<i>K. rubinsiae</i> / <i>K. xinjiangensis</i>	3	1	6	30	0.4615	46.15%
<i>K. rubinsiae</i> / <i>K. rhizosphaerae</i>	9	5	0	26	0.7826	78.26%
<i>K. rubinsiae</i> / <i>K. gynurae</i>	3	1	6	30	0.4615	46.15%
<i>K. rubinsiae</i> / <i>K. aurantiacus</i>	7	8	2	23	0.5833	58.33%
<i>K. rubinsiae</i> / <i>K. radiotolerans</i>	ND	ND	ND	ND	ND	ND
<i>K. rubinsiae</i> / <i>K. vitellinus</i>	ND	ND	ND	ND	ND	ND
<i>K. rubinsiae</i> / <i>K. indalonis</i>	ND	ND	ND	ND	ND	ND
<i>K. rubinsiae</i> / <i>K. siccus</i>	ND	ND	ND	ND	ND	ND

ND, Not determined

The obtained results present the percentage of similarity compared to the second reference species (*K. vitellinus*). The species are classified in decreasing order according to the *Dice* coefficient for the fatty acids only (Table 21), which is as follows:

K. vitellinus (VI) > *K. indalonis* (IN) = *K. endophyticus* (EN) > *K. terrestris* (TE) > *K. rhizosphaerae* (RH) > *K. siccus* (SI) = *K. xinjiangensis* (XI) = *K. gynurae* (GYN) > *K. gypseus* (GYP) > *K. radiotolerans* (RA) > *K. rubinsiae* (RU) > *K. aurantiacus* (AURA) > *K. glutinatus* (GL) = *K. aureoles* (AU) > *K. mangrovi* (MA).

Table 21. Calculation of similarity by coefficient of *Dice* fatty acids.

Species	a (1;1)	b (0;1)	c (1;0)	d (0;0)	$D = \frac{2a}{2a + b + c}$	percentege
<i>K. vitellinus / K. vitellinus</i>	4	0	0	45	1	100%
<i>K. vitellinus / K. indalonis</i>	4	4	0	41	0.6666	66.66%
<i>K. vitellinus / K. siccus</i>	2	4	2	41	0.4	40%
<i>K. vitellinus / K. rubinsiae</i>	3	12	1	33	0.3157	31.57%
<i>K. vitellinus / K. aureoles</i>	2	8	2	37	0.2857	28.57%
<i>K. vitellinus / K. terrestris</i>	3	5	1	40	0.5	50%
<i>K. vitellinus / K. mangrovi</i>	3	15	1	30	0.2727	27.27%
<i>K. vitellinus / K. gypseus</i>	4	13	0	32	0.3809	38.09%
<i>K. vitellinus / K. endophyticus</i>	4	4	0	41	0.6666	66.66%
<i>K. vitellinus / K. glutinatus</i>	3	14	1	31	0.2857	28.57%
<i>K. vitellinus / K. xinjiangensis</i>	1	0	3	45	0.4	40%
<i>K. vitellinus / K. rhizosphaerae</i>	4	9	0	36	0.4705	47.05%
<i>K. vitellinus / K. gynuraa</i>	2	4	2	41	0.4	40%
<i>K. vitellinus / K. radiotolerans</i>	2	6	2	39	0.3333	33.33%
<i>K. vitellinus / K. aurantiacus</i>	4	18	0	27	0.3076	30.76%

The *PAST 4.16c* is used to construct agglomerative Hierarchical Clustering dendograms (AHC) from the chemotaxonomic data based on the two coefficients, using the classical clustring method.

The dendrograms in Figures 15 and 17 that are based on *Jaccard* and *Dice* coefficients shows that we can classify the 12 species only because of the lack of chemotaxonomy data (sugar, amino acid, lipides, menaquinones and phospholipids).

The dendrograms in Figures 16 and 18 that are based on *Jaccard* and *Dice* coefficients show topology of 15 species classified in order of fatty acid.

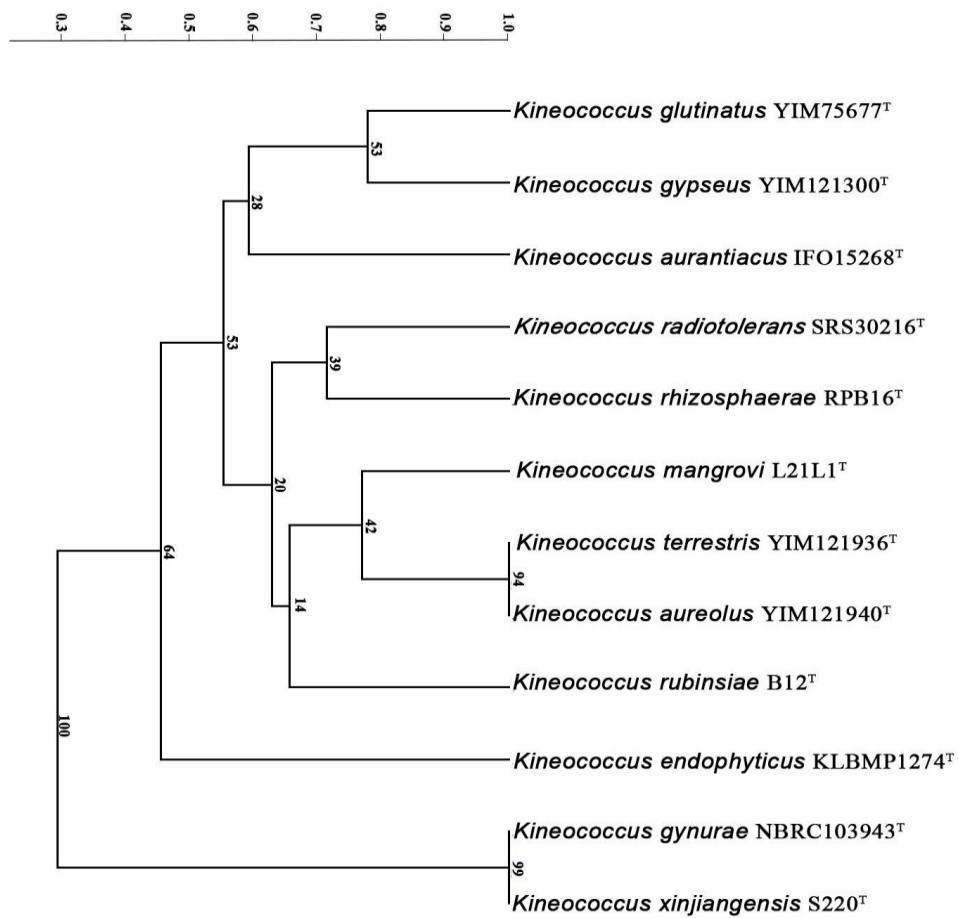


Figure 15. Chemotaxonomy dendrogram based on *Jaccard* coefficient for 12 species of *Kineococcus*, using classical clustering method.

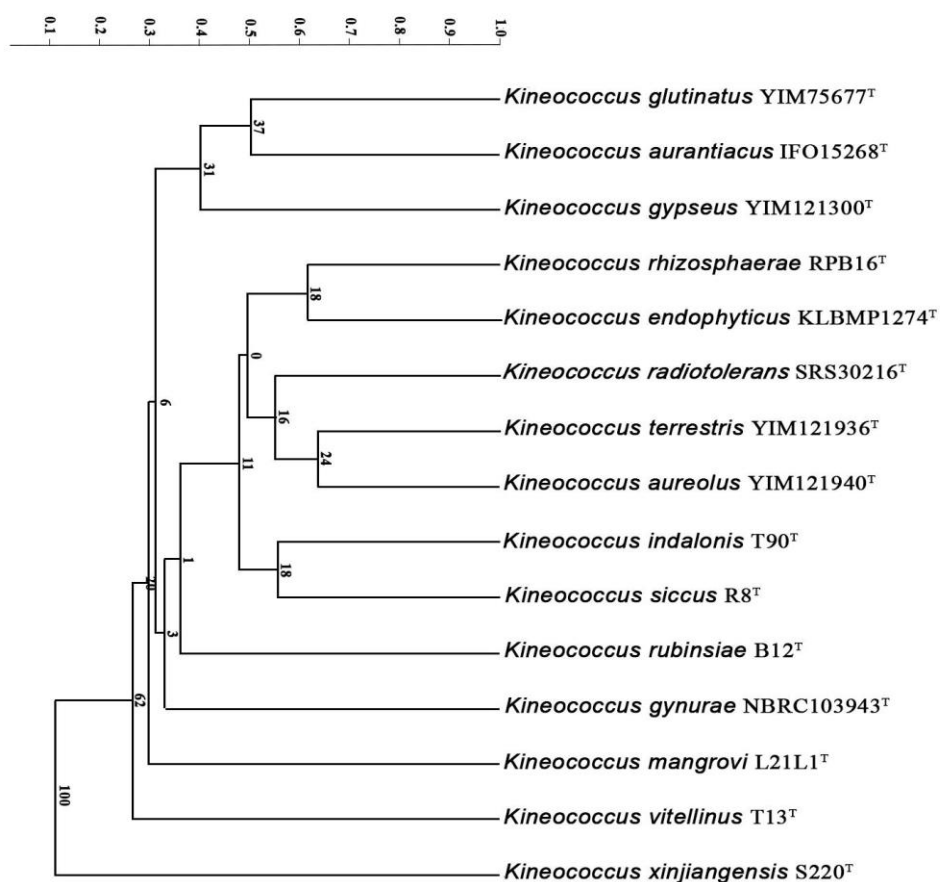


Figure 16. Chemotaxonomy dendrogram based on *Jaccard* coefficient for 15 species of *Kineococcus*, using classical clustering method.

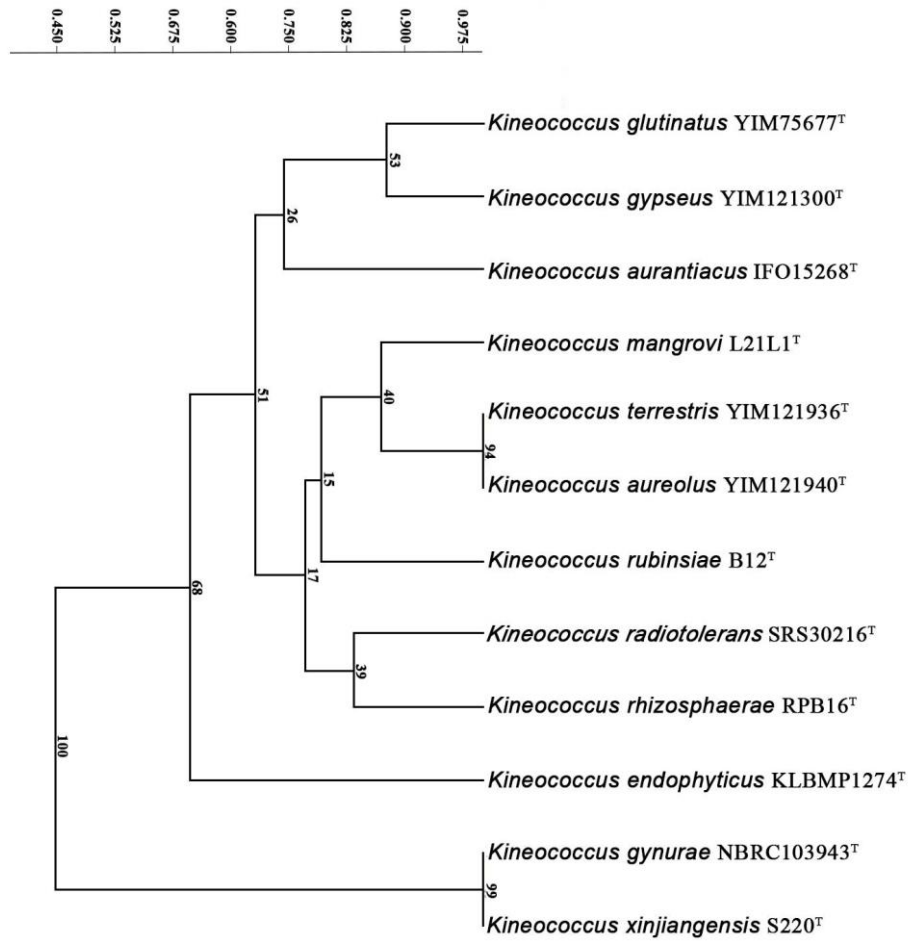


Figure 17. Chemotaxonomy dendrogram based on *Dice* coefficient, for 12 species of *Kineococcus*, using classical clustering method.

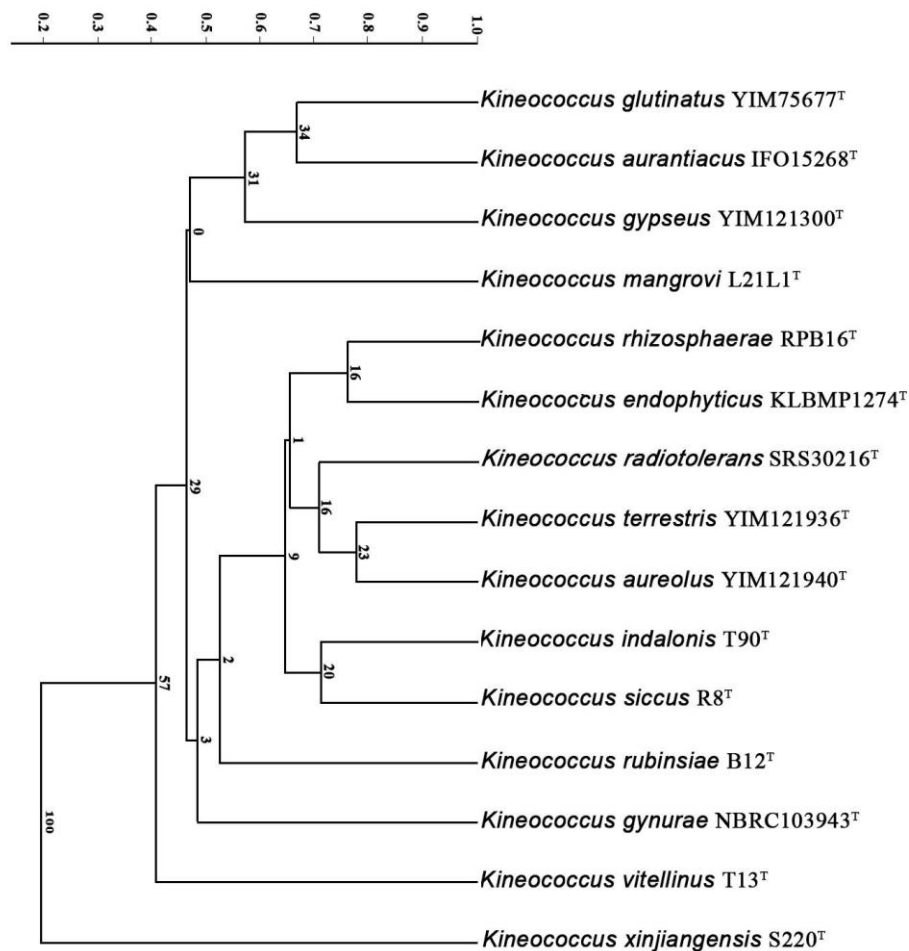


Figure 18. Chemotaxonomy dendrogram based on *Dice* coefficient for 15 species of *Kineococcus*, using classical clustering method.

3.3 Molecular analysis

In this section will have two studies which are the phylogenetic study based on a single gene (the 16S rRNA gene) and phylogenomic study based on the whole genome (the complete DNA).

3.3.1 Phylogenetic study (16S rRNA)

The 16S rRNA sequences of all the published and validated *Kineococcus* species are downloaded from a database website called EZBioCloud in FASTA format then treated by the computer program MEGA to build AHC that is shown in Figure 19.

16S rRNA gene sequences have been widely used for the identification of prokaryotes. However, the flood of sequences of non-type strains and the lack of a peer-reviewed database for 16S rRNA gene sequences of type strains have made routine identification of isolates difficult and labor-intensive.

Using inference methods available on the platform. The system developed provides users with a similarity-based search, multiple sequence alignments, and various phylogenetic analyses. All of these functions, together with the 16S rRNA gene sequence database of type strains, can be successfully used for automated and reliable identification of prokaryotic isolates (Chun *et al.*, 2007).

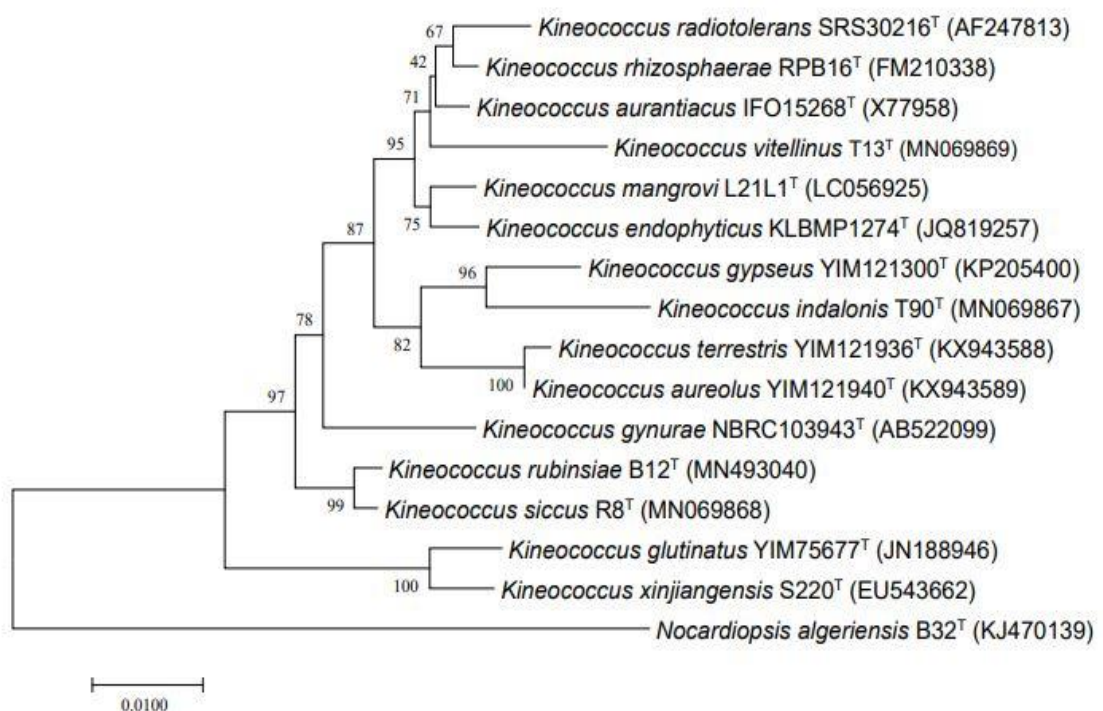


Figure 19. 16S rRNA phylogeny tree using NJ method (Saitou and Nei, 1987).

The structure of the phylogenetic tree showed a notably different arrangement compared to the one obtained from the chemotaxonomic method. Using the branch lengths provided in Table 19 and Table 21, we calculated the evolutionary distances. These distances helped establish a ranking of similarities, allowing for a comparison with the results obtained from both the chemotaxonomic analysis and EZbiocloud.

The obtained results present the percentage of similarity compared to the reference species. The species are classified in decreasing order according to the 16S rRNA (Table 22), which is as follows:

K. vitellinus (VI) > *K. aurantiacus* (AURA) > *K. radiotolerans* (RA) > *K. rhizosphaerae* (RH) > *K. endophyticus* (EN) > *K. mangrovi* (MA) > *K. siccus* (SI) > *K. aureoles* (AU) > *K. terrestris* (TE) > *K. rubinsiae* (RU) > *K. gypseus* (GYP) > *K. gynurae* (GYN) > *K. indalonis* (IN) > *K. glutinatus* (GL) > *K. xinjiangensis* (XI).

Table 22. Similarity order according to EZbiocloud

Name	Ascecion	Similarity %	Variation ratio
<i>K. vitellinus</i>	MN069869	100 %	0/1406
<i>K. aurantiacus</i>	X77958	98.48 %	21/1386
<i>K. radiotolerans</i>	CP000750	97.58 %	34/1404
<i>K. rhizosphaerae</i>	FM210338	97.57 %	34/1402
<i>K. endophyticus</i>	JQ819257	97.44 %	36/1404
<i>K. mangrovi</i>	LC056925	97.23 %	39/1406
<i>K. siccus</i>	MN069868	96.63 %	47/1395
<i>K. aureolus</i>	KX943589	96.37 %	51/1404
<i>K. terrestris</i>	KX943588	96.15 %	54/1404
<i>K. rubinsiae</i>	MN493040	96.08 %	55/1404
<i>K. gypseus</i>	KP205400	95.73 %	60/1404
<i>K. gynurae</i>	EF667339	95.57 %	58/1341
<i>K. indalonis</i>	MN069867	95.40 %	63/1395
<i>K. glutinatus</i>	JQ314347	93.97 %	84/1393
<i>K. xinjiangensis</i>	EU543662	93.53 %	89/1376

In Table 22, a comparison of similarity order between the studied species and subspecies of the genus *Kineococcus*, was carried out, and we have noticed that chemotaxonomy based on *Jaccard* coefficient has given a completely identical similarity order with *Dice* coefficient but a major difference in similarity order to EZbiocloud phylogeny. The order of similarity in the 16S rRNA phylogeny using the NJ method, is calculated from the branch's length of the phylogeny tree, and are set in the increasing order of the evolution distances.

The two phenetic trees based on the *Jaccard* and *Dice* coefficient of association, and the phylogenetic tree based on 16S rRNA gene sequences using the same clustering has given the same result.

In light of this, the unsettled taxonomic position was further examined and resolved through dDDH analysis.

3.3.2 Phylogenomic study (whole genome)

Figure 20 shows the phylogenomic tree built on the TYGS website. With genome sequencing technology, methods based on complete genome sequences

appeared, including trees based on gene content (Fitz-Gibbon and House (1999); Snel *et al.* (1999); Tekaiia *et al.* (1999); Lin and Gerstein (2000); Korbel *et al.* 2002), gene order (Korbel *et al.* 2002), average ortholog similarity (Clarke *et al.* 2002), and genome conservation, a novel genome-based method combining gene content and sequence similarity (Kunin *et al.* 2005).

It all has changed with the arrival of genome sequencing technologies. The amount of biological data began to grow quickly, as sequenced genomes became commonplace and then skyrocketed when next-generation sequencing (NGS), spread the “omics” revolution. Since January 2008, the speed of DNA sequencing is beating the infamous “Moore's law,” and all of a sudden, not only astrophysicists but also biologists are facing big data, and big data needs to be organized big time (Zhulin, 2015).

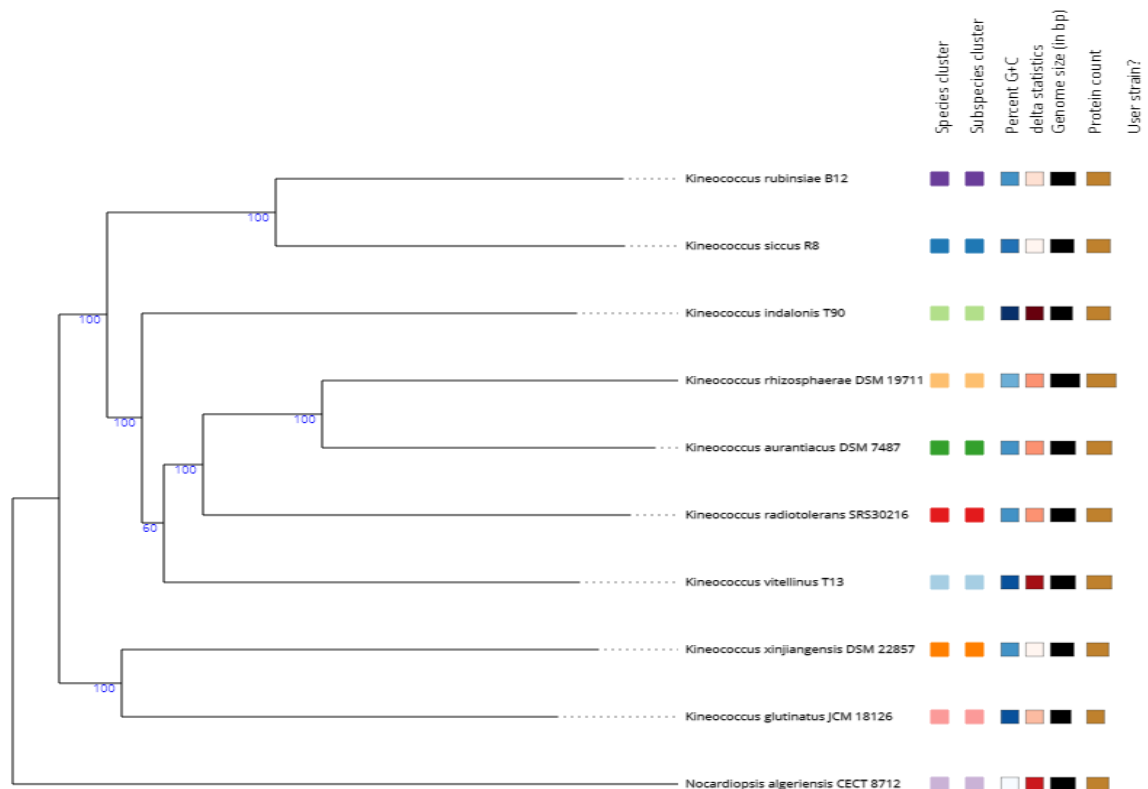


Figure 20. The phylogenomic tree of studied species of the *Kineococcus* genus is built into the TYGS website.

NCBI (The National Center for Biotechnology Information) hosts a vast collection of genomic databases, including GenBank, which stores annotated DNA

sequences, as well as databases for other types of molecular data such as protein sequences, gene expression, and genetic variation.

To build the hierarchical tree (phylogenetic tree) of the *Kineococcus* genus, we use the help of a website called TYGS, which offers a taxonomic classification of the whole genome.

The TYGS (Type (Strain) Genome Server) website hosted by DSMZ (German Collection of Microorganisms and Cell Cultures) is an online platform dedicated to providing tools and resources for microbial genomics research. It specializes in the analysis and comparison of bacterial genomes, particularly focusing on type strains, which are the original strains from which a species was first described.

The TYGS website offers various features and services, including: Genome analysis tools, Comparative genomics, Data repository and taxonomic classification.

Whole-genome similarity metrics such as Average Nucleotide Identity (ANI) help address this question by facilitating high resolution taxonomic analysis of thousands of genomes from diverse phylogenetic lineages. In Table 23 we resulted the comparison using the ANI value.

Table 23. Features of the genome sequences used in this study.

taxonomic name	Genome assembly	GenBank assembly accession number	Total length (bp)	GC content (%)	Number of proteins	OrthoANIu value (%)	Contigs
<i>K.vitellinus</i>	ASM990631v1	GCF_009906315.1	4,855,962	75.40	4648	100	698
<i>K.indalonis</i>	ASM990639v1	GCF_009906395.1	4,497,479	76.33	4350	82.00	1063
<i>K.radiotolerans</i>	ASM1730v1	GCF_000017305.1	4,956,672	74.25	4681	81.99	3
<i>K.aurantiacus</i>	ASM1340934v1	GCF_013409345.1	5,027,298	74.46	4737	81.12	2
<i>K.rhizosphaerae</i>	ASM300205v1	GCF_003002055.1	5,610,032	73.75	5371	80.24	63
<i>K.rubinsiae</i>	ASM1183980v1	GCF_011839805.1	4,880,137	74.16	4452	78.91	119
<i>K.siccus</i>	ASM990679v1	GCF_009906795.1	4,580,277	75.10	4319	78.79	495
<i>K.xinjiangensis</i>	ASM293462v1	GCF_002934625.1	4,617,682	74.65	4153	76.70	42
<i>K.mangrovi</i>	No data	No data	No data	No data	No data	No data	No data
<i>K.aureolus</i>	No data	No data	No data	No data	No data	No data	No data
<i>K.terrestris</i>	No data	No data	No data	No data	No data	No data	No data
<i>K.endophyticus</i>	No data	No data	No data	No data	No data	No data	No data
<i>K.gypseus</i>	No data	No data	No data	No data	No data	No data	No data
<i>K.gynurae</i>	No data	No data	No data	No data	No data	No data	No data
<i>K.glutinatus</i>	No data	No data	No data	No data	No data	No data	No data

To complete the analysis between the methods used and mentioned previously we created a table that collects all the variables, Table 24 shows the results of different similarities order in relation to *K. vitellinus* (Molina-Menor *et al.* 2021).

Table 24. similarity order to the reference species (*K. vitellinus*) using different methods.

dHDD (%)	OrthoANIu value (%)	Δ G+C	Δ proteins	Δ base pair (bp)	16S rRNA (%)	Jaccard (%)	Dice (%)
<i>K. vitellinus</i> 100%	<i>K. vitellinus</i> 100.00%	<i>K. vitellinus</i> 0	<i>K. vitellinus</i> 0	<i>K. vitellinus</i> 0 bp	<i>K. vitellinus</i> 100%	<i>K. vitellinus</i> 100%	<i>K. vitellinus</i> 100 %
<i>K. indalonis</i> 25.60%	<i>K. indalonis</i> 82.00%	<i>K. siccus</i> 0.3	<i>K. radiotolerans</i> 33	<i>K. rubinsiae</i> 36943 bp	<i>K. aurantiacus</i> 98.48%	<i>K. indalonis</i> 50%	<i>K. indalonis</i> 66.66%
<i>K. radiotolerans</i> 25.20%	<i>K. radiotolerans</i> 81.99%	<i>K. xinjiangensis</i> 0.75	<i>K. aurantiacus</i> 89	<i>K. radiotolerans</i> 113478 bp	<i>K. radiotolerans</i> 97.58%	<i>K. endophyticus</i> 50%	<i>K. endophyticus</i> 66.66%
<i>K. aurantiacus</i> 24.20%	<i>K. aurantiacus</i> 81.12%	<i>K. indalonis</i> 0.93	<i>K. rubinsiae</i> 196	<i>K. aurantiacus</i> 184104 bp	<i>K. rhizosphaerae</i> 97.57%	<i>K. terrestris</i> 33.33%	<i>K. terrestris</i> 50%
<i>K. rhizosphaerae</i> 23.70%	<i>K. rhizosphaerae</i> 80.24%	<i>K. aurantiacus</i> 0.94	<i>K. indalonis</i> 298	<i>K. xinjiangensis</i> 225512 bp	<i>K. endophyticus</i> 97.44%	<i>K. rhizosphaerae</i> 30.76%	<i>K. rhizosphaerae</i> 47.05%
<i>K. rubinsiae</i> 22.40%	<i>K. rubinsiae</i> 78.91%	<i>K. radiotolerans</i> 1.15	<i>K. siccus</i> 329	<i>K. siccus</i> 274154 bp	<i>K. mangrovi</i> 97.23%	<i>K. siccus</i> 25%	<i>K. siccus</i> 40%
<i>K. siccus</i> 22.20%	<i>K. siccus</i> 78.79%	<i>K. rubinsiae</i> 1.24	<i>K. xinjiangensis</i> 495	<i>K. indalonis</i> 363948 bp	<i>K. siccus</i> 96.63%	<i>K. gynurae</i> 25%	<i>K. gynurae</i> 40%
<i>K. xinjiangensis</i> 20.70%	<i>K. xinjiangensis</i> 76.70%	<i>K. rhizosphaerae</i> 1.65	<i>K. rhizosphaerae</i> 723	<i>K. glutinatus</i> 746940 bp	<i>K. aureolus</i> 96.37%	<i>K. xinjiangensis</i> 25%	<i>K. xinjiangensis</i> 40%
<i>K. terrestris</i> No data	<i>K. terrestris</i> No data	<i>K. terrestris</i> No data	<i>K. glutinatus</i> No data	<i>K. rhizosphaerae</i> No data	<i>K. terrestris</i> 96.15%	<i>K. gypseus</i> 23.52%	<i>K. gypseus</i> 38.09%
<i>K. endophyticus</i> No data	<i>K. endophyticus</i> No data	<i>K. endophyticus</i> No data	<i>K. terrestris</i> No data	<i>K. terrestris</i> No data	<i>K. rubinsiae</i> 96.08%	<i>K. radiotolerans</i> 20%	<i>K. radiotolerans</i> 33.33%
<i>K. mangrovi</i> No data	<i>K. mangrovi</i> No data	<i>K. mangrovi</i> No data	<i>K. endophyticus</i> No data	<i>K. endophyticus</i> No data	<i>K. gypseus</i> 95.73%	<i>K. rubinsiae</i> 18.75%	<i>K. rubinsiae</i> 31.57%

<i>K. gypseus</i> No data	<i>K. gypseus</i> No data	<i>K. gypseus</i> No data	<i>K. mangrovi</i> No data	<i>K. mangrovi</i> No data	<i>K. gynurae</i> 95.67%	<i>K. aurantiacus</i> 18.18%	<i>K. aurantiacus</i> 30.76%
<i>K. gynurae</i> No data	<i>K. gynurae</i> No data	<i>K. gynurae</i> No data	<i>K. gypseus</i> No data	<i>K. gypseus</i> No data	<i>K. indalonis</i> 95.48%	<i>K. aureoles</i> 16.66%	<i>K. aureoles</i> 28.57%
<i>K. glutinatus</i> No data	<i>K. glutinatus</i> No data	<i>K. glutinatus</i> No data	<i>K. gynurae</i> No data	<i>K. gynurae</i> No data	<i>K. glutinatus</i> 93.97%	<i>K. glutinatus</i> 16.66%	<i>K. glutinatus</i> 28.57%
<i>K. aurantiacus</i> No data	<i>K. aureoles</i> No data	<i>K. aureoles</i> No data	<i>K. aureoles</i> No data	<i>K. aureoles</i> No data	<i>K. xinjiangensis</i> 93.53%	<i>K. mangrovi</i> 15.78%	<i>K. mangrovi</i> 27.27%

Considering the limitations outlined in chemotaxonomy, as well as the observations by Embley *et al.* (1988) and Stackebrandt and Schumann (2006) regarding the vast diversity within the *Actinobacteria* phylum, it is prudent to approach numerical chemotaxonomy results with caution. Despite the significant value of chemotaxonomic analysis in distinguishing *Kineococcus* or any other *Actinobacteria* at the genus level (Goodfellow *et al.*, 2012), this includes analyses based on *Jaccard* and *Dice* coefficients applied to the 15 *Kineococcus* species. Efforts to correlate these findings with 16S rRNA phylogeny using the NJ method have revealed persistent discrepancies. While chemotaxonomy has shown some utility, phylogenetic analysis is, in fact, more reliable, though it is not infallible. Nonetheless, it has demonstrated a surprising alignment between ANI and dDDH factors, suggesting its potential effectiveness in elucidating taxonomic relationships within *Kineococcus* species.

Table 24 shows the difference in results and accuracy between chemotaxonomy and new approaches based on whole-genome-wide analyses. Given the scarcity of data and inconclusive results in the case of the last three species published, *K. vitellinus*, *K. indalonis*, and *K. siccus*, regarding cellular components (sugars, amino acids, menaquinones, polar lipids), it shows the trend towards reliance on molecular methods.

CHAPTER IV: CONCLUSION & PERSPECTIVES

While there is consistency between the chemotaxonomy analysis based on *Jaccard* and *Dice* coefficients, the results from the 16S rRNA molecular approach using the NJ method are not identical. Due to the limited availability of chemotaxonomic data, especially for certain species within this genus, it is prudent to interpret these results cautiously. Therefore, this comparative study should be expanded to include more thoroughly chemically studied genera and a larger sample size to ensure statistical significance. Future research should also explore additional statistical coefficients for enhanced consistency. Moreover, it is advisable not to rely solely on the NJ phylogeny method, and other methods should be considered in the study.

Although chemotaxonomic methods have been enormously important in the past with identification and classification schemes, it remains to be seen in what form they will be utilized in the genomic era, and the suite of methods available in the era of omics. In the future, with the knowledge of genes and related biochemical metabolism derived from genomic studies, researchers or related taxonomic journals should be encouraged to include this information in taxonomic descriptions (Vandamme and Sutcliffe, 2021).

Besides the traditional polyphasic approach, numerous additional tools can enhance the classification and identification of this genus. by correlating experimental data with genomic information. By integrating artificial intelligence, machine learning, and neural networks, we can achieve more precise phenotypic and chemotaxonomic predictions in prokaryotic taxonomy research. The confidence in *in silico* modeling of biomarkers can be improved; however, we still in need of chemotaxonomic analysis for the declaration of species.

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ANNEXES

Annexe I

16S rRNA gene sequences of the studied species of *Kineococcus*, and *Nocardiopsis_algeriensis*.1. *Kineococcus glutinatus* YIM 75677 JN188946

GTTTGCCTGGCTCAGGACGAAGCAAGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAACCCCTTCGGGGGG
 GATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTGGCTCTGGGATAACTCCGGGAAACCGGAGCT
 AATACCGGATACGACTCACCCCGGCATCGGGTGTGGGTGGAAAGTTTTTCGGCTGGGGATGGGCTCGCGGCCAT
 CAGCTTGTGGTGGGGTATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG
 GACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGCGAAAAGCCTGACGCAG
 CGACGCCGCTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGCAAGTGAACGGTACC
 TGCAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGTGAAGCGTTGACCGGAATTAT
 TGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAAACCTCAGGGCTTAACCCCTGAGCTTGCAGTGG
 GTACGGGCAGACTAGAGTGCAGTGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGG
 AACACCGGTGGCGAAGGCGGGTCTCTGGGCCGTAACGTGACGCTGAGGAGCGAAAGCATGGGGAGCGAaCAGGATTA
 GATACCCCTGgTAGTCCATGCCGTAACGTTGGGCGCTAGGTGTGGGACTCATTCCACGAGTCTGTGCCGAGCTA
 ACGCATTAAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGCCCCGACAAG
 CGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCGCAGTGCACCGGCAG
 AGATGTCGGGTCAATTTAGGTGGCTGCGTGCAGGTGGTGCATGGTTGTGCTGAGCTCGTGTGCTGAGATGTTGGGT
 TAAGTCCCAGCAACGAGCGCAACCCCTCGTTCTATGTTGCCAGCGCGTCATGGCGGGGACTCATAGGAGACTGCCGG
 GGTCAACTCGGAGGAGGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCACGCATGCTACAA
 TGGACGGTACAAAGGGCTGCGAGACCGTGAAGTGGAGCGAATCCAAAAAGCCGTCCTCAGTTCGGATCGGGGTC
 TGCAACTCGACCCCGTGAAGTCCGAGTGCCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGC
 CTTGTACACACCGCCCGTACGTCACGAAAGTCCGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGA
 GCCGTCAAGGTGGGACTGGCGATTGGGACGAAGTCTTACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATCAC
 C

2. *Kineococcus gynurae* NBRC 103943 AB522099

GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAAGCCCTTCGGGGTGGATCAGTGGCGAACGG
 GTGAGTAACACGTGAGCAACCTGCCCTGGCTCTGGGATAACCACCGGAAACGGTGGCTAATACTGGATGTGACG
 CCTGCACGCATGTGCTGGGTGTGGAAAGATTTTTTCGGCTGGGGATGGGCTCGCGGCCATCAGCTTGTGGTGGG
 GTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCC
 CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACCCGCGTGAGG
 GATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCTTCCGGTGAACGGTACCTGCAGAAGAAGCACC
 GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCT
 CGTAGGCGGTGTGTCGCTGTGTAACCTCAGGGCTTAACTCTGAGCTTGCAGTGGGTACGGGCATACTTG
 AGTGTGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAA
 GCGGGTCTCTGGGCGATTACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTC
 CATGCCGTAaACGTTGGGCGCTAGGTGTGGGGTCCATTCCACGGATTCCGTGCCGAGCTAACGCATTAAGCGCCC
 CGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGGGCCCCGACAAGCGGCGGAGCATGCGG
 ATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCGCAGTGAATCCAGAGATGGGGTGTCAAT
 TAGTTGGTTGCGTACAGGTGGTGCATGGTTGTGCTGAGTGCCTGCTGAGATGTTGGGTAAAGTCCCAGCAACGA
 GCGCAACCCCTCGTTCCATGTTGCCAGCACTTCGGGTGGGACTCATGGGAGACTGCCGGGTCAACTCGGAGGAA
 GGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCGTTACAGAGGG
 CTGCGATACCGTGAAGTGGAGCGAATCCCTTAAAGCTGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGT
 GAAGTCCGAGTGCCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
 GTCACGTGATGAAAGTCCGTAACACCCGAAGCCGGTGGCCTAACCCCTTGTGGGGGGAGCCGTCAAGGTGGGACT
 GGCGATTGGGACGAAGTCTTACAAGGTAGCCGTACCGGAAGGTGC

3. *Kineococcus gypseus* YIM 121300 KP205400

CTGCCCTTCAGAGTTTGCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGA
 GCCCTTCGGGGTGGATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTCAGCTCTGGGACAACCACT
 GGAAACGGTGGCTAATACCGGATACGACCCGTCTCGGCATCGAGTGCGGGTGGAAAGAAGTTCGGCTGGGGATGG
 GCTCGCGGCCATCAGCTTGTGGTGGGGTATGGCCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGA
 CCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTGTCAATGGGCGC

AAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCCA
TCTTTCGGGGTGGTGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGG
GTGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTGTGTGCGCTCTGCTGTGAAAACCTCAGG
GCTCAACTCTGAGCTTGCAGTGGGTACGGGCACACTTGAGTGCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGG
TGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGTAACCTGACGCTGAgGAGCGAA
AgcATGGGGAgCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTGTGGGGTCCATT
CCACGGACTCCGTGCCGCAGCTAACGCATTAAGCGCCCCGCTGGGGAGTACGGCCGAAGGCTAAAACTCAAAG
GAATTGACGGGGGCCCCGACAAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCT
TGACATGCACGGTGCAGTCCCAGAGATGGGACGTCATTTAGGTGGTTCGTGCACAGGTGGTGCATGGTTGTGCTCA
GCTCGTGTGCTGAGATGTTGGGTTAAGTCCCAGCAACGAGCGCAACCCCTCGTTCCATGTTGCCAGCAGTTTCGGCTG
GGGACTCATGGGAGACTGCCGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTC
TTGGGCTTCACGCATGCTACAATGGCCAGTACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCT
GGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTTCGGAGTTCGCTAGTAATCGCAGATCAGCAACGC
TGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTACGTCATGAAAGTTCGGTAACACCCGAAGCCGGTG
GCCAACCCTGACGGGGGGAGCTGTGCAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTAC
CGGAAGGTGcGGCTGGATcACCTCCTaAGGGCAGCTTGGCGTAATT

4. *Kineococcus indalonis* T90 MN069867

TTTGATTATGGCTCAGGACGAACGCTGGCGGGCTGCTTAACACATGCAAGTCGAACGGGTGAACCCCTTCGGGGGG
ATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTCAGCTCCGGGACAACCACTGGAAACGGTGGCTA
ATACCGGATACGACCCGCTCGGGCATCCGACGCGGGTGGAAAGTCTTTTCGGCTGGGGATGGGCTCGCGGCCAT
CAGCTTGTGGTGGGGTGTGGCCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG
GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAG
CGACGCCGCTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCCGCGCTCCGGTGGCG
TGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGAACGGTGT
CCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTGTGTGCGCTCTGCTGTGAAAACCTCAGGGCTCAACTCTGAG
CTTGCAGTGGGTACGGGCACACTTGAGTGCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGA
TATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAAGCATgGGGAGCG
AacAGGATTAgATAACCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTGTGGGGTCCATTCCACGGACTCCGT
GCCGCAGCTAACGCATTAAGCGCCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGG
CCCCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCACGGT
GGCGCTCCAGAGATGGAGCTTCATTTAGGTGGCCGTGCACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTGCTGA
GATGTTGGGTTAAGTCCCAGCAACGAGCGCAACCCCTCGTTCCATGTTGCCAGCGCGTGTGAGCGGGGACTCATGGG
AGACTGCCGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGCTTGGGCTTCAGC
CATGCTACAATGGCCAGTACAGAGGGCTGCGATACCGCGAGGTGGAGCGAATCCCAAAAAGCTGGTCTCAGTTCCG
GATCGGGGTCTGCAACTCGACCCCGTGAAGTTCGGAGTTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATAC
GTTCCCGGGCCTTGTACACACCGCCCGTACGTCATGAAAGTTCGGTAACACCCGAAGCCGGTGGCCCAACCCG

5. *Kineococcus mangrove* L2-1-L1 LC056925

TGGCTCAGGACGAACGCTGGCGGGCTGCTTAACACATGCAAGTCGAACGGTGATCCCTCTTCGGGGGGTGATCAG
TGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTGGCTCTGGGACAACCACTGGAAACGGTGGCTAATACC
GGATACGACCCGCGCAGGCATCTGTTGTGGGTGGAAAGTTTTTCGGCTGGGGATGGGCTCGCGGCCATCAGCTT
GTTGGTGGGGTGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGA
GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGC
CGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCTCCGAAGAAGCCGCGCTTTGTGGTGTGGTGAC
GGTAGGAGCAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGAACGGTGTGTCGG
AATTATTGGGCGTAAAGAGCTCGTAGGCGGTGTGTGCGCTCTGCTGTGAAAACCTCAGGGCTCAACTCTGAGCTTG
CAGTGGGTACGGGCACACTTGAGTGTGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATC
AGGAGAACACCGGTGGCGAAGGCGGGTCTCTGGGCGAGTTACTGACGCTGAGGAGCGAAAGCATGGGgAGCGAACA
GgATTAGATAcCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTGTGGGGTCCATTCCACGGATTCCGTGCC
CAGCTAACGCATTAAGCCCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCGCGGTGGAA
TCCCAGAGATGGGGTGTCAATTTAGTTGGTTCGCGTACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTGCTGAGATG
TTGGGTTAAGTCCCAGCAACGAGCGCAACCCCTCGTTCCATGTTGCCAGCACTTCGGGTGGGGACTCATGGGAGACT
GCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGCTTGGGCTTCACGCATGC
TACAATGGCCAGTACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCTGGTCTCAGTTCGGATCG
GGGTCTGCAACTCGACCCCGTGAAGTTCGGAGTTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCC
CGGGCCTTGTACACACCGCCCGTACGTCATGAAAGTTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGG
GGAGCTGTGCAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGA

6. *Kineococcus radiotolerans* SRS30216 AF247813

TGAGTTTTATCATGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAACCCCTTCGG
 GGGGATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTGGCTCTGGGACAACCACTGGAAACGGT
 GGCTAATACCGGATACGACTCATACCCGCATGGTGTGTGGGTGGAAAGTTTTTCGGCTGGGGATGGGCTCGCGGC
 CTATCAGCTTGTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCCGGCCACA
 CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGAT
 GCAGCGACGCCGCTGAGGGATGACGGCCTTCGGGTGTAAACCTCTTTCAGCTCCGAAGAAGCGAAAGTGACGG
 TAGGAGCAGAAGAAGCACCAGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGTGAAGCGTTGTCCGGAA
 TTATTGGGCGTAAAGAGCTCGTAGGCGGTGTGTGCGCTCTGCTGTGAAAACCTCAGGGCTCAACTCTGAGCTTGCA
 GTGGGTACGGGCACACTTGAGTGCTGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAG
 GAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCAGTTACTGACGctGAGGAGCGAAAGCATGGGGAgcGAACAGG
 AttAGATACccTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTGTGGGGTCCATTCCACGGATTCCGTGCCGCA
 GCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGGCCCGCA
 CAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCGCGGTGGAATC
 CCAGAGATGGGGTGTCAATTTAGTTGGTTCGCGTACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTGAGATGTT
 GGGTTAAGTCCCAGCAACGAGCGCAACCCTCGTTCCATGTTGCCAGCACGTTATGGTGGGGACTCATGGGAGACTG
 CCGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCACGCATGCT
 ACAATGGCCAGTACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCTGGTCTCAGTTCCGATCGG
 GGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCC
 GGGCCTTGACACACCGCCCCGTA

7. *Kineococcus rhizosphaerae* RP-B16 FM210338

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGATCCCTTTCGGGGGGTGATCAGTGGC
 GAACGGGTGAGTAACACGTGAGCAACCTGCCCTGGCTCTGGGACAACCACTGGAAACGGTGGCTAATACCGGAT
 ACGACCTACCTCGGCATCGAGTGTGGGTGGAAAGTTTTTCGGCTGGGGATGGGCTCGCGGCCTATCAGCTTGTG
 GTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCCGGCCACACTGGGACTGAGACA
 CGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCG
 TGAGGGATGACGGCCTTCGGGTGTAAACCTCTTTCAGCTCCGAAGAAGCCGCACTTGTGGTGTGGTGACGGTA
 GGAGCAGAAGAAGCACCAGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGTGAAGCGTTGTCCGGAATT
 ATTGGGCGTAAAGAGCTCGTAGGCGGTGTGTGCGCTCTGCTGTGAAAACCTCAGGGCTCAACTCTGAGCTTGCAGT
 GGGTACGGGCACACTTGAGTGCTGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGA
 GGAACACCGGTGGCGAAGGCGGGTCTCTGGGCAGTTACTGACGCTGAGGAGCGAAAGCATGGGGAGCGaACAGGAT
 TagATACCCtgGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTGTGGGGTCCATTCCACGGATTCCGTGCCGAGC
 TAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGGCCCGCACA
 AGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCGCGGTGGAATCCC
 AGAGATGGGGTGTCAATTTAGTTGGTTCGCGTACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTGAGATGTTGG
 GTTAAGTCCCAGCAACGAGCGCAACCCTCGTTCCATGTTGCCAGCACGTTGATGGTGGGGACTCATGGGAGACTGCC
 GGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCACGCATGCTAC
 AATGGCCAGTACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCTGGTCTCAGTTCCGATCGGGG
 TCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGG
 GCCTTGACACACCGCCCCGTACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCGTAA

8. *Kineococcus rubinsiae* B12 MN493040

AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAAGCCCTTCGG
 GGTGGATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTGGCTCTGGGATAACCATCGGAAACGGT
 GGCTAATACTGGATACGACCCACGATCGCATGGTGTGTGGGTGGAAAGTTTTTCGGCTGGGGATGGGCTCGCGGC
 CTATCAGCTTGTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCCGGCCACA
 CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGCGAAAGCCTGAC
 GCAGCGACGCCGCTGAGGGATGACGGCCTTCGGGTGTAAACCTCTTTCAGCAGGGGAAGAAGCGAAAGTGACGG
 TACCTGCAGAAGAAGCACCAGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGTGAAGCGTTGTCCGGAA
 TTATTGGGCGTAAAGAGCTCGTAGGCGGTGTGTGCGCTCTGCTGTGAAAACCTCAGGGCTTAACCTGAGCTTGCA
 GTGGGTACGGGCGAGACTTGAGTGGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAG
 GAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCGTAACTGACGCTGAGGAGCGAAAGCATGGGGAGcGAACAGG
 AttAGATACccTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTGTGGGGTCCATTCCACGGATTCCGTGCCGCA
 GCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGGCCCGCA
 CAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCGCGGTGGAATC
 CCAGAGATGGGGTGTCAATTTAGTTGGTTCGCGTACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTGAGATGTT

GGTTAAGTCCCACAACGAGCGCAACCCTCGTTCCATGTTGCCAGCACGTTATGGTGGGACTCATGGGAGACTG
 CCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTCACGCATGCT
 ACAATGGCCAGTACAGAGGGCTGCGATACCGCGAGGTGGAGCGAATCCCAAAAAGCTGGTCTCAGTTCGGATCGG
 GGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCC
 GGGCCTTGACACACCGCCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCTCTTGTAGGG
 GGGAGCCGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGA
 TCACCT

9. *Kineococcus siccus* R8 MN069868

TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAAGCCCTTCGGGGTGGATCAGTG
 GCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTGGCTCTGGGATAACCATCGGAAACGGTGGCTAATACTGG
 ATACGACCCACGATCGCATGGTGTGTGGGTGGAAAGTTTTTCGGCTGGGGATGGGCTCGCGGCCATCAGCTTGT
 TGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGA
 CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGCGAAAAGCCTGACGCAGCGACGCCG
 CGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAAGTACGGTACCTGCAGAA
 AAGCACCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGTGAAGCGTGTCCGGAATTATTGGGCGTA
 AAGAGCTCGTAGGCGGTTTTGTGCGCTCTGCTGTGAAAACCTCAGGGCTTAACTCTGAGCTTGCAGTGGGTACGGC
 AGACTTGAGTGCAGTGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGG
 TGGCGAAGGCGGGTCTCTGGGCCGTAACGACGCTGAGGAGCGAAAAGCATGGGGAGCGAACAGGATTAATACCCCT
 GGTAGTCCATGCGTAACGTTGGGCGCTAGGTGTGGGGTCCATTCCACGGATTCCGTGCCGAGCTAACGCATTA
 AGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGCCCCGACAAGCGGCGGAG
 CATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCGCGGTGGAATCCAGAGATGGGG
 TGTCATTTAGTTGGTTCGCGTACAGGTGGTGCATGGTTGTGCTGAGCTCGTGTGCTGAGATGTTGGGTAAAGTCCC
 GCAACGAGCGCAACCCTCGTTCCATGTTGCCAGCAATTCGGTTGGGGACTCATGGGAGACTGCCGGGGTCAACTC
 GGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCAGTA
 CAGAGGGCTGCGATACCGTGAAGTGGAGCGAATCCCAAAAAGCTGGTCTCAGTTCGGATCGGGGTCTGCAACTCG
 ACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACAC
 ACCGCCCCGTACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACC

10. *Kineococcus terrestris* YIM 121936 KX943588

AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAAGCCCTTCGG
 GGTGGATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTGGCTCTGGGACAACCACTGGAAACGGT
 GGCTAATACCGGATACGACTCGCCCCGGCATCGGGTGCGGGTGGAAAGATTTTCATCGGCTGGGGATGGGCTCGCG
 GCCTATCAGCTTGTGGTGGGGTGATGGCCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCA
 CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTG
 ATGCAGCGACCCGCGTGAAGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCTTTGACGAAGCCCTTCGGGG
 TGACGGTAGGAGCAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGTGAAGCGTTGT
 CCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTGTGTGCGCTCTGCTGTGAAAACCTCAGGGCTCAACTCTGAG
 CTTGACAGTGGGTACGGGCACACTTGAGTGCAGTGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGA
 TATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGTAACGACGCTGAGGAGCGAAAAGCATGGGGAGCG
 AAcAGGGTTAgATACCCCTGGTAGTCCATGCCGTAACGTTGGGCGCTAGGTGTGGGACCCATTCCACGGGTTCCGT
 GCCGAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGG
 CCCGACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCGCGGT
 GGCGCCTCAGAGATGGGGCTTCATTTAGGTGGTGCAGCACAGGTGGTGCATGGTTGTGCTGAGCTCGTGTGCTGA
 GATGTTGGGTTAAGTCCCACAACGAGCGCAACCCTCGTTCCATGTTGCCAGCAGTTCGGCTGGGACTCATGGGA
 GACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCACGC
 ACGTACAATGACCACTACAGAGGGCTGCGATACCGTGAAGTGGAGCGAATCCCAAAAAGCTGGTCTCAGTTCGG
 ATCGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACG
 TTCCCGGGCCTTGTACACACCGCCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTG
 TGGGAGGGAGTCGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTA

11. *Kineococcus vitellinus* T13 MN069869

CTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAACCCCTCCTCGGAGGGGGATCA
 GTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTGGCTCTGGGACAACCACTGGAAACGGTGGCTAATAC
 CGGATACGACCCACCTCGGCATCGGGTGTGGGTGGAAAGTTTTTCGGCTGGGGATGGGCTCGCGGCCATCAGCT
 TGTGGTGGGGTGATGGCCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTG
 AGACACGGCCAGACTCATAACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAAGCCTGATGCAGCGACG
 CCGCGTGAAGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCTCCGAAGAAGCGCAAGTACGGTGGAGCAG

AAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTGTCCGGAATTATTGGGC
 GTAAAGAGCTCGTAGGCGGTGTGTGCGCTCTGCTGTGAAAACCTCAGGGCTCAACTCTGAGCTTGCAGTGGGTACG
 GGCACACTTGAGTGTGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACAC
 CGGTGGCGAAGGCGGGTCTCTGGGCAGTTACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATAGATAC
 CctGGTAGTccATGCCGTAAACGTTGGGGCGCTAGGTGTGGGGTCCATCCACGGATTCCGTGCCCGAGCTAACGCAT
 TAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGGCCCGACAAGCGGCGG
 AGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCGCGGTGGAATCCAGAGATGG
 GGTGTCAATTTAGTTGGTTCGCGTACAGGTGGTGCATGGTTGTGCTGAGCTCGTGTGCTGAGATGTTGGGTAAAGTC
 CCGCAACGAGCGCAACCCTCGTTCCATGTTGCCAGCAATTCGGTTGGGGACTCATGGGAGACTGCCGGGTCAAC
 TCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTACCGCATGCTACAATGGCCAG
 TACAGAGGGCTGCGATACCGCGAGGTGGAGCGAATCCCAAAAAGCTGGTCTCAGTTCGGATCGGGGTCTGCAACT
 CGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCCGGGTGAGTAACACGTGGCCTTGTAC
 ACACCGCCCGTACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCTAACCCGCAAGGGA

12. *Kineococcus_xinjiangensis* S2-20 EU543662

TGCAAGTCGAACGGTGAACCCCTTCGGGGGGGATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCT
 GGCTCTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATATGACCTGTACCCGCATGGTGCAGGGTGGAAAAG
 TTTTTCGGCTGGGGATGGGCTCGCGGCCATCAGCTTGTGGTGGGGTAATGGCCTACCAAGGCAGCAGCGGGTA
 GCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGA
 ATATTGCGCAATGGGCGAAAGCCTGACGCAGCGACGCCGCGTGGGGATGACGGCCTTCGGGTGTAAACCTCTT
 TCAGCAGGGAAGAAGCGAAAGTGCAGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGTAA
 TACGTAGGGTGAACGCTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAA
 AACTCAGGGCTTAACCCCTGAGCTTGCAGTGGGTACGGGCAGACTAGAGTGCAGGTAGGGGAGACTGGAATTCCTGG
 TGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGTAACCTGACGCTG
 AGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACCCCTGGTAGTCCATGCCGTAAACGTTGGGGCctAGGTGTG
 GggCTCATTccACGAGTTCTGTGCCGAGCTAACGCATTAAGCGCCCCGCTGGGGAGTACGGCCGCAAGGCTAAA
 ACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTA
 CCAAGGCTTGACATGCGCAGTGGACCGGCAGAGATGTGGGTCATTTAGTTGGCTGCGTGCAGGTGGTGCATGGT
 TGTGCTCAGCTCGTGTGCTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTCGTTCTATGTTGCCAGCGC
 GTTATGGCGGGGACTCATAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCC
 CCTTATGTCTTGGGCTTACGCATGCTACAATGGACGGTACAAAGGGCTGCGAGACCGTGAGGTGGAGCGAATCC
 CAAAAGCCGTCCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGAT
 CAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCCGCCCCGTCACGTCACGAAAGTCGGTAACACCCG
 AAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGC

13. *Kineococcus_aurantiacus* IFO 15268 X77958

GGCGTGCTTAACACATGCAAGTCGAACGGTGAACCCCTTCGGGGGGATCAGTGGCGAACGGGTGAGTAACACGTG
 AGCAACCTGCCCTGGCTCTGGGACAACCCTGGAACCGGTGGCTAATACCGGATACGACCTGCCTCGGCATCGA
 GTGCGGGTGGAAAGTTTTTCGGCTGGGGATGGGCTCGCGGCCATCAGCTTGTGGTGGGGTAATGGCCTACCAA
 GGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGA
 GGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGGGGATGACGGCCTTCGG
 GTTGTAAACCTCTTTCAGCTCCGAAGAAGCGCAAGTGCAGGTAGGAGCAGAAGAAGCACCGGCTAACTACGTGCC
 AGCAGCCGCGGTAATACGTAGGGTGAACGCTTGTCCGGAATTATTGGGGCGTAAAGAGCTCGTAGGCGGTGTGT
 CGCGTCTGCTGTGAAAACCTCAGGGCTCAACTCTGAGCTTGCAGTGGGTACGGGCACACTTGAGTGTGTAGGGGA
 GACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGG
 CAGTTACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACCCCTGGTAGTCCATGCGcTAAACGT
 TggGCGCTAggTGTGGGGTCCATTCCACGGATTCCGTGCCGACGTAACGCATTAAGCGCCCCGCTGGGGAGTAC
 GGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCA
 ACGCGAAGAACCTTACCAAGGCTTGACATGCGCGGTGGAATCCAGAGATGGGGNGTCATTTAGTTGGTGCAGTA
 CAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGCTGAGATGTTGGGTTAAGTCCCAGACGAGCGCAACCCCTCGTT
 CCATGTTGCCAGCACTTGCAGGTGGGGACTCATGGGAGACTGCCGGGTCAACTCGGAGGAGTGGGGATGACG
 TCAAATCATCATGCCCTTATGTCTTGGGCTTACCGCATGCTACAATGGCCAGTACAGAGGGCTGCGATACCCG
 AGGTGGAGCGAATCCCAAAAAGCTGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCC
 CTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGCCCTTGTACACACCCGCCCCGTCACGTCATGAAA
 GTCGGTAACACCCGAACCGGTGGCCCAACCCGTCAGGGGGANNNGTCAAGGTGGGACGTGGCGA

14. *Kineococcus aureoles* YIM 121940 KX943589

AGAGTTTGGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAAGCCCTTCGG
GGTGGATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCCTGGCTCTGGGACAACCACTGGAAACGGT
GGCTAATACCGGATACGACTCGCCCCGGCATCGGGTGGCGGGTGGAAAGATTTTCATCGGCTGGGGATGGGCTCGCG
GCCTATCAGCTTGTGGTGGGGTGATGGCCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCA
CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTG
ATGCAGCGACGCCGCTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCTTTGACGAAGCCCTCGGGG
TGACGGTAGGAGCAGAAGAAGCACCAGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGGTGAAGCGTTGT
CCGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTGTGTCGCGTCTGCTGTGAAAACCTCAGGGCTCAACTCTGAG
CTTGCAAGTGGGTACGGGCACACTTGAGTGCAGTGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGA
TATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAAGCArGGGAGCG
AacAGGATTAgATACCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTGTGGGACCCATTCCACGGGTTCCGT
GCCGAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCGCGGT
GGCGCCTCAGAGATGGGGCTTCATTTAGGTGGTTCGCGCACAGGTGGTGCATGGTTGTGCTGAGCTCGTGTGCTGA
GATGTTGGGTTAAGTCCCAGCAACGAGCGCAACCCCTCGTTCCATGTTGCCAGCAGTTCGGCTGGGGACTCATGGGA
GACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCACGC
ATGCTACAATGGCCAGTACAGAGGGCTGCGATAACCGTGAGGTGGAGCGAATCCCAAAAAGCTGGTCTCAGTTCGG
ATCGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACG
TTCCCGGGCCTTGTACACACCGCCCGTACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTG
TGGGAGGGAGTCTGCAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTA

15. *Kineococcus endophyticus* KLBMP 1274 JQ819257

GCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAAGCCCTTCGGGGTGGATCAGTGGC
GAACGGGTGAGTAACACGTGAGCAACCTGCCCCCTGGCTCTGGGACAACCACTGGAAACGGTGGCTAATACCGGAT
ACGACCCGCACAGGCATCTGTTGTGGGTGGAAAGTTTTTCGGCTGGGGATGGGCTCGCGGCCATCAGCTTGTG
GTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCCACTGGGACTGAGACA
CGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACCGCCG
TGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCTCCGAAGAAGCGCAAGTGCAGGTAGGAGCAGAAGAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGAAGCGTTGTCCGGAATTATTGGGCGTAAA
GAGCTCGTAGGCGGTGTGTGCGCTCTGCTGTGAAAACCTCAGGGCTCAACTCTGAGCTTGCAGTGGGTACGGGCAC
ACTTGAGTGTGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTG
GCGAAGGCGGGTCTCTGGGCAGTTACTGACGCTGAGGAGCGAAAGCaTGGGGAGCGAACAGGATTAGatACCCTGG
TAgTCCATGccGTAACGTTGGGCGCTAGGTGTGGGGTCCATTCCACGGATTCCGTGCCGAGCTAACGCATTAAG
CGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGGCCCGCACAAGCGGGCGGAGCA
TGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCGCGGTGGAATCCAGAGATGGGGTG
TCATTTAGTTGGTTCGCTACAGTGGTGCATGGTTGTGCTGAGTTCGCTGAGTGTGGGTTAAGTCCCGC
AACGAGCGCAACCCCTCGTTCCATGTTGCCAGCAGTGTGGGGACTCATGGGAGACTGCCGGGTCAACTCG
GAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCAGTAC
AGAGGGCTGCGATAACCATGAGGTGGAGCGAATCCCAAAAAGCTGGTCTCAGTTCGGATCGGGGTCTGCAACTCGA
CCCTGTGAAGTTCGGAGTTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACA
CCGCCCGTACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCGTAAGGGGGGGAGCTGTGCAAGG
TGGGACTGGCGATTGGGACGAAGTCG

16. *Nocardiosis algeriensis* B32 KJ470139

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGGCCCTTCGGGGTACACGAGCGGCGA
ACGGGTGAGTAACACGTGAGCAACCTGCCCCCTGACTCCGGGATAAGCGGGCGAAACGCCGTCTAATACCGGATAC
GACCTCCTGTCTCATGGCGGGGGGTGGAAAGTTTTTTTCGGTTGGGGATGGGCTCGCGGCCATCAGCTTGTGGT
GGGGTAACGGCCTACCAAGGCGATTACGGGTAGCCGGCCTGAGAGGGCGACCGGCCCACTGGGACTGAGACACG
GCCAGACTCCTGCGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGCGAAAGCCTGACGCAGCGACGCCGCGTG
GGGGTACAGCCGCTTCGGGTTGTAAACCTCTTTTACCACCAACGAGGCTCCGGGTTCTCTCGGGGTTGACGGTA
GGTGGGGAAATAAGGACCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGGTCCGAGCGTTGTCCGGAATT
ATTGGGCGTAAAGAGCTCGTAGGCGGCATGTTGCGTCTGCTGTGAAAGACCGGGGCTTAACTCCGGTTCTGCAGT
GGATACGGCATGCTAGAGGTAGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGA
GGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCTTACCTGACGCTGAGGAGCGAAAGCATGGGGAGCGaACAGGAT
TAgATACCCrGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTGTGGGGACTTTCCACGGTTTCCGCGCCGTAGCT
AACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGGCCCGCACA

GCGGCGGAGCATGTTGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGTTTGACATCACCCGTGGACCTGCA
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GGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCAAACATGCTACA
ATGGCCGGTACAATGGGCGTGCGATACCGCAAGGTGGAGCGAATCCCTAAAAGCCGGTCTCAGTTCGGATTGGGG
TCTGCAACTCGACCCCATGAAGGTGGAGTCGCTAGTAATCGCGGATCAGCAACGCCGCGGTGAATACGTTCCCGG
GCCTTGTACACACCGCCCGTCACGTTCATGAAAGTCGGCAACACCCGAAACTTGTGGCCTAACCTTCGGGGAGGG
AGTGAGTGAAGGTGGGGCTGGCGATTGGGACGA

Annexe II

Matrix of similarity and distance calculated by Past 4.16c based on coefficient of *Jaccard* for characteristics without fatty acids.

Similarity and distance indices

	<i>K. aurantia</i>	<i>K. radiotole</i>	<i>K. gynurae</i>	<i>K. rhizosph</i>	<i>K. xinjiange</i>	<i>K. glutinatu</i>	<i>K. endophy</i>	<i>K. gypseus</i>	<i>K. mangrov</i>	<i>K. terrestris</i>	<i>K. aureolus</i>	<i>K. rubinsiae</i>
<i>K. aurantia</i>	1	0.38888889	0.26666667	0.52631579	0.26666667	0.57142857	0.36842105	0.61111111	0.66666667	0.55555556	0.55555556	0.41176471
<i>K. radiotole</i>	0.38888889	1	0.16666667	0.71428571	0.16666667	0.47368421	0.4	0.6	0.53846154	0.64285714	0.64285714	0.58333333
<i>K. gynurae</i>	0.26666667	0.16666667	1	0.28571429	1	0.22222222	0.36363636	0.28571429	0.4	0.30769231	0.30769231	0.3
<i>K. rhizosph</i>	0.52631579	0.71428571	0.28571429	1	0.28571429	0.6	0.47058824	0.64705882	0.6	0.6875	0.6875	0.64285714
<i>K. xinjiange</i>	0.26666667	0.16666667	1	0.28571429	1	0.22222222	0.36363636	0.28571429	0.4	0.30769231	0.30769231	0.3
<i>K. glutinatu</i>	0.57142857	0.47368421	0.22222222	0.6	0.22222222	1	0.45	0.77777778	0.47368421	0.55	0.55	0.42105263
<i>K. endophy</i>	0.36842105	0.4	0.36363636	0.47058824	0.36363636	0.45	1	0.5625	0.4	0.5	0.5	0.42857143
<i>K. gypseus</i>	0.61111111	0.6	0.28571429	0.64705882	0.28571429	0.77777778	0.5625	1	0.6	0.6875	0.6875	0.53333333
<i>K. mangrov</i>	0.66666667	0.53846154	0.4	0.6	0.4	0.47368421	0.4	0.6	1	0.76923077	0.76923077	0.58333333
<i>K. terrestris</i>	0.55555556	0.64285714	0.30769231	0.6875	0.30769231	0.55	0.5	0.6875	0.76923077	1	1	0.69230769
<i>K. aureolus</i>	0.55555556	0.64285714	0.30769231	0.6875	0.30769231	0.55	0.5	0.6875	0.76923077	1	1	0.69230769
<i>K. rubinsiae</i>	0.41176471	0.58333333	0.3	0.64285714	0.3	0.42105263	0.42857143	0.53333333	0.58333333	0.69230769	0.69230769	1

Matrix of similarity and distance calculated by Past 4.16c based on coefficient of *Jaccard* for fatty acids characteristics.

Similarity and distance indices

	<i>K. aurantia</i>	<i>K. radiotole</i>	<i>K. gynurae</i>	<i>K. rhizosph</i>	<i>K. xinjiange</i>	<i>K. glutinatu</i>	<i>K. endophy</i>	<i>K. gypseus</i>	<i>K. mangrov</i>	<i>K. terrestris</i>	<i>K. aureolus</i>	<i>K. rubinsiae</i>	<i>K. vitellinus</i>	<i>K. indalonis</i>	<i>K. siccus</i>
<i>K. aurantia</i>	1	0.36363636	0.2173913	0.59090909	0.04545454	0.5	0.36363636	0.44444444	0.37931034	0.36363636	0.39130435	0.42307692	0.18181818	0.30434783	0.27272727
<i>K. radiotole</i>	0.36363636	1	0.16666667	0.5	0.125	0.25	0.33333333	0.19047619	0.3	0.6	0.5	0.27777778	0.2	0.45454545	0.4
<i>K. gynurae</i>	0.2173913	0.16666667	1	0.35714286	0.16666667	0.21052632	0.55555556	0.21052632	0.26315789	0.4	0.23076923	0.3125	0.25	0.27272727	0.33333333
<i>K. rhizosph</i>	0.59090909	0.5	0.35714286	1	0.07692307	0.36363636	0.61538462	0.30434783	0.40909091	0.61538462	0.53333333	0.47368421	0.30769231	0.5	0.46153846
<i>K. xinjiange</i>	0.04545454	0.125	0.16666667	0.07692307	1	0.05882352	0.125	0.05882352	0.05555556	0.125	0.06666667	0.25	0.125	0.16666667	0.16666667
<i>K. glutinatu</i>	0.5	0.25	0.21052632	0.36363636	0.05882352	1	0.31578947	0.36	0.2962963	0.31578947	0.28571429	0.28	0.16666667	0.31578947	0.27777778
<i>K. endophy</i>	0.36363636	0.33333333	0.55555556	0.61538462	0.125	0.31578947	1	0.31578947	0.36842105	0.6	0.38461538	0.35294118	0.5	0.6	0.4
<i>K. gypseus</i>	0.44444444	0.19047619	0.21052632	0.30434783	0.05882352	0.36	0.31578947	1	0.25	0.31578947	0.28571429	0.33333333	0.23529412	0.31578947	0.21052632
<i>K. mangrov</i>	0.37931034	0.3	0.26315789	0.40909091	0.05555556	0.2962963	0.36842105	0.25	1	0.3	0.2173913	0.22222222	0.15789474	0.3	0.26315789
<i>K. terrestris</i>	0.36363636	0.6	0.4	0.61538462	0.125	0.31578947	0.6	0.31578947	0.3	1	0.63636364	0.4375	0.33333333	0.6	0.55555556
<i>K. aureolus</i>	0.39130435	0.5	0.23076923	0.53333333	0.1	0.28571429	0.38461538	0.28571429	0.2173913	0.63636364	1	0.38888889	0.16666667	0.38461538	0.45454545
<i>K. rubinsiae</i>	0.42307692	0.27777778	0.3125	0.47368421	0.06666667	0.28	0.35294118	0.33333333	0.22222222	0.4375	0.38888889	1	0.1875	0.27777778	0.3125
<i>K. vitellinus</i>	0.18181818	0.2	0.25	0.30769231	0.25	0.16666667	0.5	0.23529412	0.15789474	0.33333333	0.16666667	0.1875	1	0.5	0.25
<i>K. indalonis</i>	0.30434783	0.45454545	0.27272727	0.5	0.125	0.31578947	0.6	0.31578947	0.3	0.6	0.38461538	0.27777778	0.5	1	0.55555556
<i>K. siccus</i>	0.27272727	0.4	0.33333333	0.46153846	0.16666667	0.27777778	0.4	0.21052632	0.26315789	0.55555556	0.45454545	0.3125	0.25	0.55555556	1

Annexe II

Matrix of similarity and distance calculated by Past 4.16c based on coefficient of *Dice* for characteristics without fatty acids.

Similarity and distance indices

	<i>K. aurantia</i>	<i>K. radiotole</i>	<i>K. gynurae</i>	<i>K. rhizosph</i>	<i>K. xinjiang</i>	<i>K. glutinatu</i>	<i>K. endophy</i>	<i>K. gypseus</i>	<i>K. mangrov</i>	<i>K. terrestris</i>	<i>K. aureolus</i>	<i>K. rubinsiae</i>
<i>K. aurantia</i>	1	0.56	0.42105263	0.68965517	0.42105263	0.72727273	0.53846154	0.75862069	0.8	0.71428571	0.71428571	0.58333333
<i>K. radiotole</i>	0.56	1	0.28571429	0.83333333	0.28571429	0.64285714	0.57142857	0.75	0.7	0.7826087	0.7826087	0.73684211
<i>K. gynurae</i>	0.42105263	0.28571429	1	0.44444444	1	0.36363636	0.53333333	0.44444444	0.57142857	0.47058824	0.47058824	0.46153846
<i>K. rhizosph</i>	0.68965517	0.83333333	0.44444444	1	0.44444444	0.75	0.64	0.78571429	0.75	0.81481481	0.81481481	0.7826087
<i>K. xinjiang</i>	0.42105263	0.28571429	1	0.44444444	1	0.36363636	0.53333333	0.44444444	0.57142857	0.47058824	0.47058824	0.46153846
<i>K. glutinatu</i>	0.72727273	0.64285714	0.36363636	0.75	0.36363636	1	0.62068966	0.875	0.64285714	0.70967742	0.70967742	0.59259259
<i>K. endophy</i>	0.53846154	0.57142857	0.53333333	0.64	0.53333333	0.62068966	1	0.72	0.72	0.57142857	0.66666667	0.66666667
<i>K. gypseus</i>	0.75862069	0.75	0.44444444	0.78571429	0.44444444	0.875	0.72	1	0.75	0.81481481	0.81481481	0.69565217
<i>K. mangrov</i>	0.8	0.7	0.57142857	0.75	0.57142857	0.64285714	0.57142857	0.75	1	0.86956522	0.86956522	0.73684211
<i>K. terrestris</i>	0.71428571	0.7826087	0.47058824	0.81481481	0.47058824	0.70967742	0.66666667	0.81481481	0.86956522	1	1	0.81818182
<i>K. aureolus</i>	0.71428571	0.7826087	0.47058824	0.81481481	0.47058824	0.70967742	0.66666667	0.81481481	0.86956522	1	1	0.81818182
<i>K. rubinsiae</i>	0.58333333	0.73684211	0.46153846	0.7826087	0.46153846	0.59259259	0.6	0.69565217	0.73684211	0.81818182	0.81818182	1

Matrix of similarity and distance calculated by Past 4.16c based on coefficient of *Dice* for fatty acids characteristics.

Similarity and distance indices

	<i>K. aurantia</i>	<i>K. radiotole</i>	<i>K. gynurae</i>	<i>K. rhizosph</i>	<i>K. xinjiang</i>	<i>K. glutinatu</i>	<i>K. endophy</i>	<i>K. gypseus</i>	<i>K. mangrov</i>	<i>K. terrestris</i>	<i>K. aureolus</i>	<i>K. rubinsiae</i>	<i>K. vitellinus</i>	<i>K. indalonis</i>	<i>K. siccus</i>
<i>K. aurantia</i>	1	0.53333333	0.35714286	0.74285714	0.08695652	0.66666667	0.53333333	0.61538462	0.55	0.53333333	0.5625	0.59459459	0.30769231	0.46666667	0.42857143
<i>K. radiotole</i>	0.53333333	1	0.28571429	0.66666667	0.22222222	0.4	0.5	0.32	0.46153846	0.75	0.66666667	0.43478261	0.33333333	0.625	0.57142857
<i>K. gynurae</i>	0.35714286	0.28571429	1	0.52631579	0.28571429	0.34782609	0.71428571	0.34782609	0.41666667	0.57142857	0.375	0.47619048	0.4	0.42857143	0.5
<i>K. rhizosph</i>	0.74285714	0.66666667	0.52631579	1	0.14285714	0.53333333	0.76190476	0.46666667	0.58064516	0.76190476	0.69565217	0.64285714	0.47058824	0.66666667	0.63157895
<i>K. xinjiang</i>	0.08695652	0.22222222	0.28571429	0.14285714	1	0.11111111	0.22222222	0.11111111	0.10526316	0.22222222	0.18181818	0.125	0.4	0.22222222	0.28571429
<i>K. glutinatu</i>	0.66666667	0.4	0.34782609	0.53333333	0.11111111	1	0.48	0.52941176	0.45714286	0.48	0.44444444	0.4375	0.28571429	0.48	0.43478261
<i>K. endophy</i>	0.53333333	0.5	0.71428571	0.76190476	0.22222222	0.48	1	0.48	0.53846154	0.75	0.55555556	0.52173913	0.66666667	0.75	0.57142857
<i>K. gypseus</i>	0.61538462	0.32	0.34782609	0.46666667	0.11111111	0.52941176	0.48	1	0.4	0.48	0.44444444	0.5	0.38095238	0.48	0.34782609
<i>K. mangrov</i>	0.55	0.46153846	0.41666667	0.58064516	0.10526316	0.45714286	0.53846154	0.4	1	0.46153846	0.35714286	0.36363636	0.27272727	0.46153846	0.41666667
<i>K. terrestris</i>	0.53333333	0.75	0.57142857	0.76190476	0.22222222	0.48	0.75	0.48	0.46153846	1	0.77777778	0.60869565	0.5	0.75	0.71428571
<i>K. aureolus</i>	0.5625	0.66666667	0.375	0.69565217	0.18181818	0.44444444	0.55555556	0.44444444	0.35714286	0.77777778	1	0.56	0.28571429	0.55555556	0.625
<i>K. rubinsiae</i>	0.59459459	0.43478261	0.47619048	0.64285714	0.125	0.4375	0.52173913	0.5	0.36363636	0.60869565	0.56	1	0.31578947	0.43478261	0.47619048
<i>K. vitellinus</i>	0.30769231	0.33333333	0.4	0.47058824	0.4	0.28571429	0.66666667	0.38095238	0.27272727	0.5	0.28571429	0.31578947	1	0.66666667	0.4
<i>K. indalonis</i>	0.46666667	0.625	0.42857143	0.66666667	0.22222222	0.48	0.75	0.48	0.46153846	0.75	0.55555556	0.43478261	0.66666667	1	0.71428571
<i>K. siccus</i>	0.42857143	0.57142857	0.5	0.63157895	0.28571429	0.43478261	0.57142857	0.34782609	0.41666667	0.71428571	0.625	0.47619048	0.4	0.71428571	1

*Annexe III*Molecular analysis of different species of *Kineococcus* by using EZbio cloud

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


Select hits by database **All** Valid names only Excel FASTA EzEditor2





















Tasks	Hit taxon name	Hit strain name	Accession	Similarity	Variation ratio	Completeness (%)
	Kineococcus vitellinus	T13(T)	MN069869	100.00	0/1406	97.7
	Kineococcus aurantiacus	IFO 15268(T)	X77958	98.48	21/1386	96.7
	Kineococcus radiotolerans	SRS30216(T)	CP000750	97.58	34/1404	100.0
	Kineococcus rhizosphaerae	RP-B16(T)	FM210338	97.57	34/1402	97.4
	Kineococcus endophyticus	KLBMP 1274(T)	JQ819257	97.44	36/1404	100.0
	Kineococcus mangrovi	L2-1-L1(T)	LC056925	97.23	39/1406	100.0
	Kineococcus siccus	R8(T)	MN069868	96.63	47/1395	96.9
	Kineococcus	YIM 121940(T)	KX943589	96.37	51/1404	100.0
	Kineococcus	YIM 121936(T)	KX943588	96.15	54/1404	100.0
	Kineococcus	B12(T)	MN493040	96.08	55/1404	100.0


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Annexes

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Tasks	Hit taxon name	Hit strain name	Accession	Similarity	Variation ratio	Completeness (%)
 	<i>Kineococcus gypseus</i>	YIM 121300(T)	KP205400	95.73	60/1404	100.0
 	<i>Kineococcus gynurae</i>	KKD096(T)	EF667339	95.67	58/1341	93.2
 	<i>Kineococcus indalonis</i>	T90(T)	MN069867	95.48	63/1395	97.1
 	<i>Kineococcus glutinatus</i>	YIM 75677(T)	JQ314347	93.97	84/1393	99.2
 	EU132684_s	FFCH1107	EU132684	93.53	86/1330	92.5
 	<i>Kineococcus xinjiangensis</i>	S2-20(T)	EU543662	93.53	89/1376	96.1
 	<i>Kineosporia succinea</i>	JCM 9957(T)	BBIE01000478	93.08	97/1402	100.0
 	<i>Kineosporia mikuniensis</i>	NBRC 16234(T)	AB377117	92.87	100/1402	100.0
 	<i>Kineosporia rhamnosa</i>	JCM 9954(T)	AB003935	92.83	100/1395	100.0
 	<i>Kineosporia mesophila</i>	YIM 65293(T)	FJ214362	92.79	99/1374	98.1

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Genomique analysis of different species of *Kineococcus* by usening TYGS server

TYGS ID	Kind	Species cluster	Subspecies cluster	Preferred name	Deposit	Authority	Other deposits	Synonymous taxon names	Base pairs	Percent G+C
121502	type strain	1	0	<i>Kineococcus vitellinus</i>	T13	Molina-Menor et al. 2021	CECT 9936; DSM 110024	<i>Kineococcus vitellinus</i>	4,843,194	75.41
121504	type strain	2	1	<i>Kineococcus siccus</i>	R8	Molina-Menor et al. 2021	CECT 9937; DSM 110025	<i>Kineococcus siccus</i>	4,569,040	75.13
121506	type strain	3	2	<i>Kineococcus indalonis</i>	T90	Molina-Menor et al. 2021	CECT 9938; DSM 110026	<i>Kineococcus indalonis</i>	4,479,246	76.35
13598	type strain	4	3	<i>Kineococcus aurantiacus</i>	DSM 7487	Yokota et al. 1993	CIP 105426; ATCC 51238; JCM 10180; IFO 15268; NBRC 15268; VKM Ac-1947; RA 333	<i>Kineococcus aurantiacus</i>	5,027,298	74.46
148740	type strain	5	4	<i>Kineococcus glutinatus</i>	JCM 18126	Nie et al. 2012	DSM 26692; NBRC 111526; CCTCC AA 209075; YIM 75677	<i>Kineococcus glutinatus</i>	4,096,254	75.4
155	type strain	6	5	<i>Kineococcus glutinatus</i>	SRS30216	Phillips et al. 2002 emend.	ATCC BAA-149; DSM 14245; JCM	<i>Kineococcus glutinatus</i>	4,956,672	74.25

Annexes

TCGC TYGS TTGG		TYPE (STRAIN) GENOME SERVER		Submit your query	News	Examples ▾	Background	FAQ	Feedback	API	DSMZ Digital Diversity		DSMZ
148740	type strain	5	4	<i>Kineococcus glutinatus</i>	JCM 18126	Nie et al. 2012	NBRC 111526; CCTCC AA 209075; YIM 75677	<i>Kineococcus glutinatus</i>	4,096,254	75.4			
155	type strain	6	5	<i>Kineococcus radiotolerans</i>	SRS30216	Phillips et al. 2002 emend. Nouioui et al. 2018	ATCC BAA-149; DSM 14245; JCM 12686; NBRC 101839	<i>Kineococcus radiotolerans</i>	4,956,672	74.25			
22668	type strain	7	6	<i>Kineococcus rhizosphaerae</i>	DSM 19711	Lee 2009	KCTC 19366; JCM 16541; RP-B16	<i>Kineococcus rhizosphaerae</i>	5,610,032	73.75			
22864	type strain	8	7	<i>Kineococcus xinjiangensis</i>	DSM 22857	Liu et al. 2009 emend. Nouioui et al. 2018	KCTC 19474; JCM 16219; CCTCC AB 207179; S2-20	<i>Kineococcus xinjiangensis</i>	4,617,682	74.65			
24974	type strain	9	8	<i>Nocardiopsis algeriensis</i>	CECT 8712	Bouras et al. 2015	DSM 45462; B 32	<i>Nocardiopsis algeriensis</i>	4,806,673	71.33			
49398	type strain	10	9	<i>Kineococcus rubinsiae</i>	B12	Mhatre et al. 2021	NRRL B-65556; DSM 110506; FJII-L1-CM-PAB2	<i>Kineococcus rubinsiae</i>	4,880,137	74.16			

The binary value matrix in Excel

D2		Gal																																						
Species	Whole-cell sugar content of kineococcus								Amino acid and peptidoglycan content analysis of kineococcus								Menaquinones content analysis of kineococcus								Phospholipid content analysis of kineococcus															
	Glu	Man	Gal	Rib	Ara	Xyl	Mad	rham	Glut	Ala	Gly	GlcN	L-DAP	meso-DAP	asparagine	threonine	MK-7(H2)	MK-7(H4)	MK-8(H2)	MK-8(H4)	MK-8(H6)	MK-9(H2)	MK-9(H4)	MK-9(H6)	MK-10(H4)	PE	DPG	PI	PG	HPE	PC	PME	LPE	PGL	PIM	PL	2PL	GL	3L	
Kineococcus aurantiacus	1	1	1	1	1	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0
Kineococcus radiotolerans	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0
Kineococcus gynurae	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kineococcus rhizosphaerae	1	1	1	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0
Kineococcus xinjiangensis	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kineococcus glutinatus	1	1	1	1	1	0	0	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	1	1	0	0	0
Kineococcus endophyticus	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	1	1	0	1	1
Kineococcus gypseus	1	1	1	1	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0
Kineococcus mangrovi	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
Kineococcus terrae	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0
Kineococcus aurantiacus	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0
Kineococcus rubinsiae	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	
Kineococcus viteus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	
Kineococcus indologus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	
Kineococcus siciliensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	
Frequency	9/12	9/12	12/12	8/12	11/12	11/12	0/12	3/12	3/11	3/11	0/11	0/11	0/11	11/11	1/11	1/11	0/11	0/11	0/11	0/11	0/11	11/11	0/11	0/11	0/11	1/15	13/15	8/15	13/15	0/15	0/15	0/15	0/15	5/15	5/15	5/15	1/15	2/15	1/15	

