

Article

Genome Insights into *Kocuria* sp. KH4, a Metallophilic Bacterium Harboring Multiple Biosynthetic Gene Clusters (BGCs)

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Abstract

The genus *Kocuria* includes Gram-positive and environmentally versatile bacteria, which are of biotechnological interest due to their ability to synthesize secondary metabolites. In this study, the genome of *Kocuria* sp. KH4, isolated from alkaline mine tailings (southeastern Mexico), was sequenced and analyzed to determine its taxonomic affiliation and explore its metabolic and adaptive potential. The assembled genome showed a size of 3.89 Mb, a GC content of 73.2%, and 3609 coding genes. Phylogenomic analyses and genomic relationship indices (ANI, AAI, and dDDH) confirmed that strain KH4 represents a novel genomospecies within the genus *Kocuria*. Functional analysis revealed broad metabolic diversity, with genes associated with the transport and metabolism of amino acids, carbohydrates, and inorganic ions. A total of 165 genes linked to metal resistance and homeostasis mechanisms were identified, including ABC-type transport systems and ATPases, as well as specific genes for Fe, Ni, Zn, Cu, As, and Hg. Forty-eight genomic islands were also identified, encoding a wide variety of functions and mobile genetic elements (MGEs). Furthermore, six biosynthetic gene clusters (BGCs) involved in the production of nonribosomal peptides, type III polyketides, terpenes, and siderophores were detected, suggesting a remarkable potential for the synthesis of bioactive compounds. Taken together, the results highlight this strain as a promising source of secondary metabolites with potential applications in environmental, pharmaceutical, and industrial biotechnology, underscoring the importance of *Kocuria* genomes as natural reservoirs of new biosynthetic pathways.

Keywords: metal resistance; genome mining; secondary metabolites; genomospecies; mobile genetic elements; genomic islands



Academic Editor: Mireille Fouillaud

Received: 16 November 2025

Revised: 30 November 2025

Accepted: 5 December 2025

Published: 7 December 2025

Citation: Cuaxinque-Flores, G.; Gómez-Godínez, L.J.; Armenta-Medina, A.; Zelaya-Molina, L.X.; Ramos-Garza, J.; Aguirre-Noyola, J.L. Genome Insights into *Kocuria* sp. KH4, a Metallophilic Bacterium Harboring Multiple Biosynthetic Gene Clusters (BGCs). *Microbiol. Res.* **2025**, *16*, 255. <https://doi.org/10.3390/microbiolres16120255>

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1. Introduction

The genus *Kocuria* belongs to the phylum *Actinomycetota*, order *Micrococcales*, and family *Micrococcaceae* [1]. This group was formally proposed by Stackebrandt et al. [2], who reclassified several species previously included in the genus *Micrococcus*. This taxonomic separation was based on phylogenetic analyses of the 16S rRNA gene and differences

in the amino acid composition of peptidoglycan, which revealed consistent evolutionary divergence between the two lineages. In general, strains of this genus are characterized by being Gram-positive, non-spore-forming, catalase-positive, coagulase-negative, mesophilic cocci that typically grow in tetrads or irregular clusters. These bacteria display an aerobic or facultatively anaerobic metabolism and are nutritionally versatile, capable of utilizing a broad range of carbon sources, including amino acids, organic acids, simple sugars, and, in some cases, complex carbohydrates. Several species can also metabolize lipids and aromatic compounds [3]. Additionally, *Kocuria* strains often tolerate high salt concentrations, variable pH conditions, and limited nutrient availability [4]. According to The List of Prokaryotic Names with Standing in Nomenclature (LPSN, 2025), *Kocuria* comprises approximately 30 validly published species, isolated from environments as diverse as soils, deserts, sediments, marine systems, hypersaline lakes, plant tissues, and rhizospheres; some have even been associated with infections in immunosuppressed patients, reflecting their broad ecological plasticity [4–10].

Kocuria also exhibits remarkable metabolic versatility, expressed in the synthesis of extracellular enzymes such as amylases, lipases, proteases, and keratinases [11–13] and secondary metabolites such as carotenoid pigments, antibiotics, extracellular polymeric substances (EPSs), and phytohormones [14–16]. The enzymes responsible for producing these specialized metabolites are typically encoded within biosynthetic gene clusters (BGCs)—groups of physically linked genes that act together in substrate activation, assembly, modification, transport, and regulation of the final product [17]. Their organization enables coordinated expression and, in some cases, horizontal transfer, enhancing the ecological adaptability and biotechnological potential of *Kocuria* species [18]. Owing to this metabolic versatility and adaptive capacity, several *Kocuria* strains have been employed as key agents in bioremediation strategies targeting soil and water contaminated with polycyclic aromatic hydrocarbons (PAHs), textile dyes, heavy metals, and metalloids [11,19–21].

On the other hand, mine tailings represent one of the largest industrial waste streams globally, with an estimated annual production of between 7 and 14 billion tons [22]. These solid wastes are composed of fine particles of rock and residual minerals, with a composition dominated by silica, metal oxides, and traces of potentially toxic elements such as As, Pb, Zn, and Hg [23]. Mine tailings are home to proliferating metallotolerant microbial communities capable of withstanding high metal concentrations, low nutrient availability, and extreme pH levels through mechanisms of ion exclusion, biotransformation, metal precipitation, and biofilm formation [24,25]. These metallophilic microorganisms not only modulate the mobility and bioavailability of metals but also provide a valuable resource for environmental and biotechnology, with applications in bioremediation and biocatalysis under extreme conditions [26,27].

In this context, we hypothesize that genomic analysis of bacteria adapted to sulfide bearing tailings, particularly the genus *Kocuria*, will provide insight into the molecular mechanisms underlying their metal tolerance and their ability to biosynthesize bioactive compounds. The goal of this study was to sequence, assemble, and analyze the genome of *Kocuria* sp. KH4, a strain isolated from a mining region in southeastern Mexico, in order to determine its taxonomic affiliation, explore its potential for the production of secondary metabolites, and evaluate its adaptive and biotechnological capabilities through phylogenomic, pangenomic, and genome mining analyses.

2. Materials and Methods

2.1. Isolation of *Kocuria* Strains from Mine Tailings

During 2020, samples were collected from the upper zone of the “La Concha” sulfide bearing tailings, located 10 km southwest of Taxco city, Guerrero, in southeastern Mexico

(18°32'22" N, 99°38'09" W). These tailings were generated between the 1960s and 1970s during silver and gold extraction activities. The deposits are still unoxidized, exhibit a brownish-gray coloration, and are characterized by a high pH (~8.4), elevated carbonate concentration, and distinctly low electrical conductivity ($88 \mu\text{S cm}^{-1}$). In terms of metal content, they contain predominant concentrations of Cd ($8\text{--}780 \text{ mg kg}^{-1}$), Cu ($71.8\text{--}1320 \text{ mg kg}^{-1}$), Zn ($780\text{--}10,000 \text{ mg kg}^{-1}$), As ($1140\text{--}11,800 \text{ mg kg}^{-1}$), Fe ($6000\text{--}12,300 \text{ mg kg}^{-1}$), and Pb ($10,100\text{--}43,700 \text{ mg kg}^{-1}$) [28]. The tailing samples were serially diluted using a sterile MgSO_4 solution (10 mM) and spread onto plates containing alkalized Reasoner's 2A (R2A) agar (pH 8.0), prepared with the following composition (g L^{-1}): hydrolyzed casein, 0.25; peptone, 0.25; yeast extract, 0.5; meat extract, 0.3; glucose, 0.5; soluble starch, 0.5; tryptone, 0.5; sodium pyrophosphate, 0.03; and agar, 15.0. The pH was adjusted to 8.0 with 1 N NaOH prior to sterilization. Plates were incubated at 30 °C for 72 h. A representative colony exhibiting distinctive carotenoid pigmentation was subcultured at least three times under identical conditions until an axenic isolate was obtained.

2.2. Whole Genome Sequencing (WGS)

A pure culture of strain KH4 was sent to MicrobesNG (The BioHub Birmingham, Birmingham, UK) for genomic DNA sequencing. Between 5 and 40 μL of a cell suspension was lysed with 120 μL of TE buffer containing lysozyme and RNase A (0.1 mg/mL) and incubated for 25 min at 37 °C. Proteinase K (0.1 mg/mL) and SDS (0.5% *v/v*) were then added, and the mixture was incubated at 65 °C for 5 min. Genomic DNA was purified using an equivalent volume of SPRI beads and resuspended in 10 mM Tris-HCl (pH 8.0). DNA quantification was performed using the Quant-iT dsDNA HS kit (Thermo Fisher Scientific, Waltham, MA, USA) on an Eppendorf AF2200 plate reader. Library preparation was carried out on a Hamilton Microlab STAR automated liquid-handling system using the Nextera XT Library Prep kit (Illumina, San Diego, CA, USA), following the manufacturer's instructions with two modifications: the DNA concentration was doubled, and the PCR elongation time was extended to 45 s. Finally, sequencing was performed on an Illumina NovaSeq 6000 platform using 250 bp paired-end reads.

2.3. Genome Assembly and Functional Annotation

The quality of raw sequencing reads was evaluated using FastQC v0.12.1, and low-quality bases (Phred < 30) were trimmed with TrimGalore v0.6.4. Genome assembly was carried out with SPAdes v3.15.5 [29], while assembly completeness and contamination levels were assessed using CheckM v1.2.2 [30]. Gene prediction and annotation were performed using Prokka v1.14.6, and tRNA and rRNA genes were identified with Aragorn and Barrnap, respectively [31]. The circular genome map was generated with MGCplotter v1.0.1, based on GenBank files to visualize forward and reverse coding sequences, rRNA and tRNA genes, GC content, and GC skew. Functional gene classification was automatically assigned using the COGclassifier module v1.0.4 integrated into MGCplotter.

2.4. Phylogenomic and Pangenomic Analyses

Comparative genomic analysis was performed using the genome of strain KH4 together with 29 additional representative *Kocuria* species retrieved from the RefSeq database prior to October 2025 (Table S1). The structure of the pangenome was inferred using GET_HOMOLOGUES v2.0 [32] and visualized with anvio v8 [33], and the genes were classified as core, soft-core, shell, or cloud using parse_pangenome_matrix.pl. Phylogenetic markers were identified with GET_PHYLOMARKERS v2.8.1.4. The species phylogenetic tree was inferred using ASTRAL-IV, which accounts for discordance among gene trees and estimates branch lengths in substitutions per site, as implemented in IQ-TREE [34]. Phylogenetic dendrogram was visualized using FigTree v1.4.4. In silico DNA–DNA hybridization

(dDDH) analyses were performed with closely related strains. Average Nucleotide Identity (ANI) was calculated from MUMmer alignments (ANIm) using pyani v0.2.12 [35], while Average Amino Acid Identity (AAI) was obtained with CompareM v0.1.2.

2.5. Screening for Metal Resistance Genes (MRGs) and Genomic Islands (GIs)

A targeted search for bacterial metal(loid) resistance genes was performed in the genome of *Kocuria* sp. KH4 using a hidden Markov model (HMM)-based approach. Predicted protein sequences were analyzed with HMMER v3.4 against the MetHMMDB database. To ensure high-confidence detection, a stringent filtering criterion was applied: bit score ≥ 60 , HMM coverage $\geq 70\%$, and E-value $\leq 1 \times 10^{-10}$ [36]. Only matches meeting all three thresholds were considered valid, minimizing false positives. The detected genes were subsequently classified according to (i) the associated metal or metalloid (e.g., As, Cd, Co, Cu, Hg, Ni, Zn, Fe, Mn); (ii) the encoded functional mechanism, including efflux pumps, membrane transporters, biotransformation enzymes, metal-binding proteins, and metal-dependent redox systems; and (iii) the functional nature of the metal (essential vs. toxic). The relative abundance of each resistance category was quantified to estimate the proportional representation of each functional profile within the metal resistance gene repertoire of *Kocuria* sp. KH4. In parallel, genomic islands (GIs) were predicted using IslandViewer4 based on three search algorithms (IslandPath-DIMOB, SIGI-HMM, and IslandPick) that detect unusual patterns of nucleotide composition (deviations in G+C content, differences in oligonucleotide frequency, and atypical genetic codes) (Bertelli et al., 2017) [37].

2.6. Genome Mining to Identify Valuable Secondary Metabolites

The identification and characterization of biosynthetic gene clusters (BGCs) responsible for the production of specialized metabolites in *Kocuria* spp. strains were conducted using antiSMASH v8.0.4 (Antibiotics and Secondary Metabolite Analysis Shell) [38]. All genomes were analyzed with default parameters, enabling the KnownClusterBlast, ClusterBlast, and Pfam domain annotation modules to improve the detection accuracy and functional prediction of BGCs. This configuration allowed for the identification of canonical BGC classes (e.g., NRPS, PKS, RiPPs, terpenes, and siderophores), as well as hybrid and less-characterized cluster types. To explore BGC diversity across strains, the antiSMASH outputs were processed using BiG-SCAPE v1.1.8 (Biosynthetic Gene Similarity Clustering and Prospecting Engine). BiG-SCAPE was employed to (i) generate presence/absence matrices for all BGC classes detected in each genome and (ii) construct BGC similarity networks based on pairwise comparisons of domain architecture, gene order, copy number, and sequence identity [39]. This approach allowed BGCs to be grouped into Gene Cluster Families (GCFs), facilitating the assessment of biosynthetic potential, strain-level divergence, and putative functional redundancy within the genus. Network topologies and GCF distributions were subsequently examined to infer the degree of conservation, novelty, and diversification of specialized metabolite pathways among the analyzed *Kocuria* species.

3. Results

3.1. Functional Genomic Analysis of *Kocuria* sp. KH4

The *Kocuria* sp. strain KH4 was isolated from non-oxidized alkaline sulfide bearing tailings and was initially identified by analyzing its 16S rRNA gene. In a nutrient medium, this strain's colonies were bright reddish-orange, circular, convex with smooth edges, and had a diameter of 3 mm (Figure 1a). Its genome was sequenced and assembled, achieving 99% completeness with no evidence of contamination (0%). The assembly showed a mean sequencing coverage of $64.3\times$ and consisted of 51 contigs, with an N50 of 180.2 kb and

an L50 of 8 contigs. The largest contig reached 499.9 kb. The genome has a total size of 3,890,285 base pairs (bp) and contains 3609 protein-coding sequences (CDSs), 50 tRNA genes, 5 rRNA genes, and a GC content of 73.2% (Figure 1b).

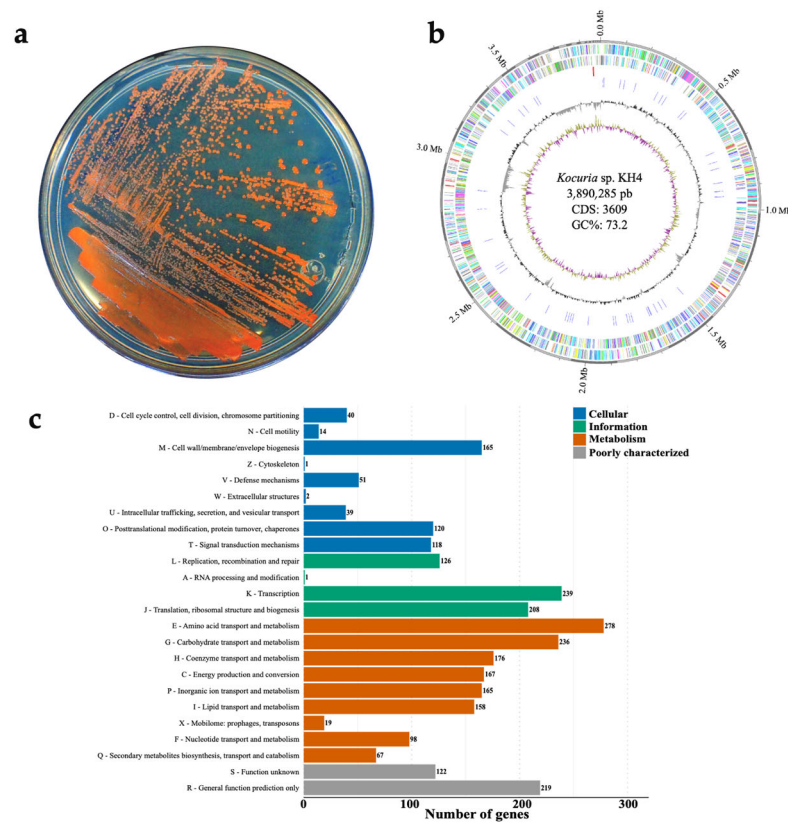


Figure 1. Phenotypic and genotypic characteristics of *Kocuria* sp. strain KH4. (a) Colonies of *Kocuria* sp. strain KH4 grown on tryptic soy agar (TSA). (b) Circular visualization of the genome. Genome size (outermost circle), genes on the positive and negative strands (2nd and 3rd circles), tRNA genes (4th circle), rRNA genes (5th circle), GC content (6th circle), and GC skew (7th circle). (c) Classification of protein-coding sequences (CDSs) according to COG categories.

Functional genome analysis, based on the Clusters of Orthologous Genes (COG) database, revealed broad metabolic diversity, with 78.39% of the predicted CDSs assigned to different functional categories (Figure 1c). The most represented categories were E (amino acid transport and metabolism, 278 CDSs), K (transcription, 239 CDSs), and G (carbohydrate transport and metabolism, 236 CDSs). Other notable categories included J (translation, ribosomal structure, and biogenesis, 208 CDSs), H (coenzyme transport and metabolism, 176 CDSs), and C (energy production and conversion, 167 CDSs). Likewise, a high representation of category P (inorganic ion transport and metabolism) was observed, with 165 CDSs associated with metal homeostasis mechanisms, which are analyzed in detail in a later section.

3.2. Genome-Based Taxonomy for *Kocuria* sp. KH4

To establish the taxonomic assignment of *Kocuria* sp. KH4, its assembled genome was analyzed using the GTDB Toolkit (GTDB-Tk). The results confirmed that KH4 belongs to the phylum *Actinomycetota*, class *Actinomycetes*, order *Actinomycetales*, family *Micrococcaceae*, and genus *Kocuria*, with *K. turfanensis* (GCF_001580365.1) being the closest species. For a more detailed approach, a phylogenomic comparison was performed using 29 reference genomes from the genus *Kocuria*. Sixty-seven CDSs shared by all genomes (core genome) were identified and used to construct the phylogenomic tree. This analysis revealed that

Kocuria sp. KH4 belongs to an independent clade. The closest phylogenetic neighbors were *K. sediminis* JCM 17929^R, *K. turfanensis* HO-9042^R, and *K. oceani* FXJ8.057^R (Figure 2). Furthermore, Overall Genome Relatedness Indices (OGRIs) were estimated, which are considered standard parameters for the delimitation of bacterial species. The average nucleotide identity (ANI) between strain KH4 and its closest relatives was below the species threshold (95%): 94.4% with *K. sediminis* JCM 17929^R, 94.1% with *K. turfanensis* HO-9042^R, and 93.4% with *K. oceani* FXJ8.057^R, with coverage greater than 73% in all cases. Consistently, the average amino acid identity (AAI) values were also below the interspecific threshold, with 93.0%, 92.4%, and 91.9% for *K. sediminis*, *K. turfanensis*, and *K. oceani*, respectively (Figure 3). Lastly, the in silico DNA–DNA hybridization (dDDH) values were below the species demarcation threshold (70%), with 54.9% for *K. sediminis* JCM 17929^R, 52.1% for *K. turfanensis* HO-9042^R, and 49.6% for *K. oceani* FXJ8.057^R. Collectively, the phylogenomic analyses and OGRIs (ANI, AAI, and dDDH) confirm that isolate KH4 is a novel genomospecies within the genus *Kocuria*.

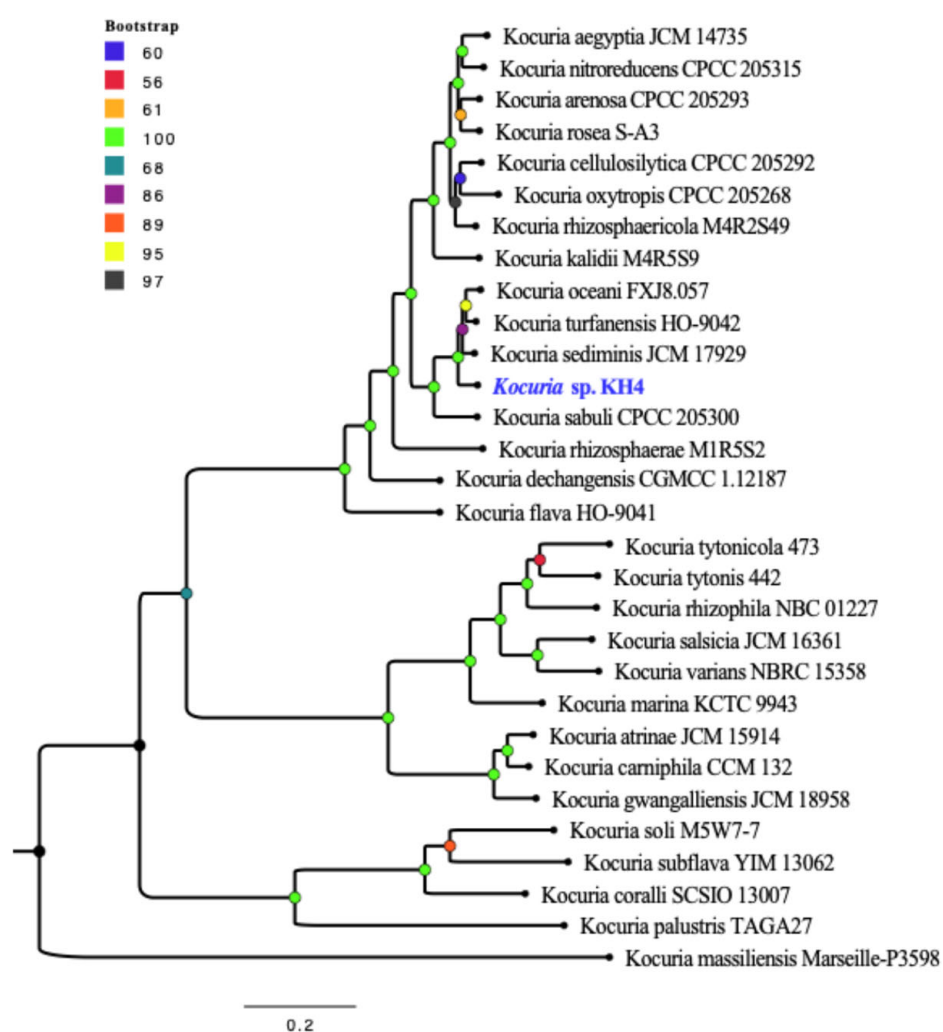


Figure 2. Phylogenomic tree with 30 genomes of the genus *Kocuria*, with 67 major single-copy genes using a GTR+F+ASC+R6 model. Strain in blue correspond to *Kocuria* sp. KH4. The color scale represents the value of the bootstraps associated with each clade.

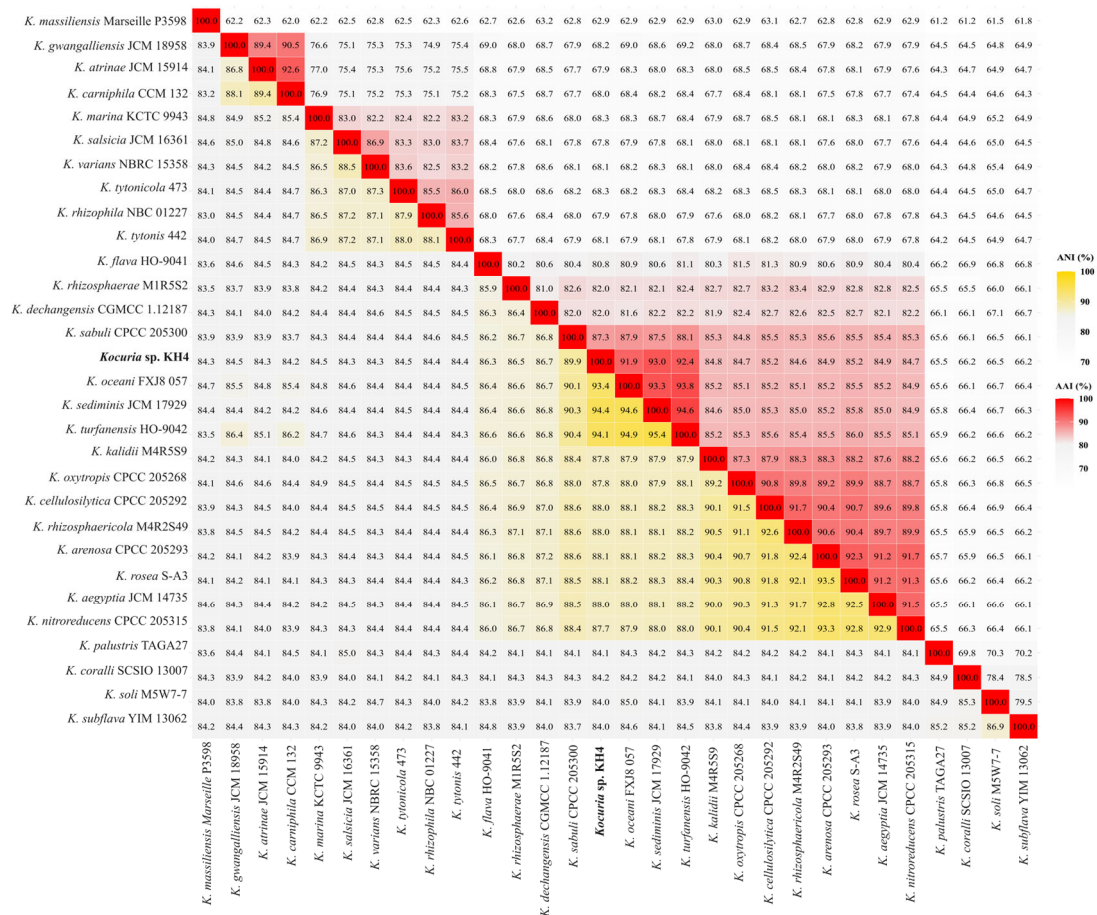


Figure 3. Heatmap showing the values (%) of average nucleotide identity (ANI) and average amino acid identity (AAI) between strains of the genus *Kocuria*. A higher color strength indicates a high degree of sequence similarity. The results for *Kocuria* sp. KH4 are highlighted in bold.

3.3. Pangenome of *Kocuria* Genus

The pangenome, which is the total set of genes present in a group of genomes, was analyzed for the *Kocuria* genus using 30 strains belonging to different species. The results revealed an open pangenome consisting of 16,913 gene clusters, indicating a high capacity to incorporate new functions as more genomes are added. On average, 129 new gene families were incorporated with each additional genome without reaching a saturation point, a trend characteristic of expanding pangenomes (Figure 4a). Conversely, the core genome curve shows a marked decrease in shared genes as the number of included genomes increases, stabilizing at around 730 orthologous groups (Figure 4b). Regarding the accessory genome, the strains were found to carry between 106 and 508 unique genes (Figure 4c). Notably, strain KH4 was found to have 276 non-shared genes (Figure 4d). These mainly encode hypothetical proteins but also elements with functions potentially relevant for environmental adaptation. These include genes encoding Hin-type recombinases and invertases and multiple transposases from the IS5, IS481, and IS1421 families, as well as the ParB protein and the nucleoid-associated protein Lsr2. Genes linked to transcriptional regulation (such as RpoD and an HTH-type regulator) were also identified, in addition to genes associated with redox metabolism (e.g., the Alx modulator), amino acid transport (YhdG), and various dehydrogenases and methyltransferases involved in specialized metabolic pathways. Taken together, these results suggest that the *Kocuria* genus has a highly diverse gene repertoire, likely due to its wide ecological distribution and ability to adapt to different niches.

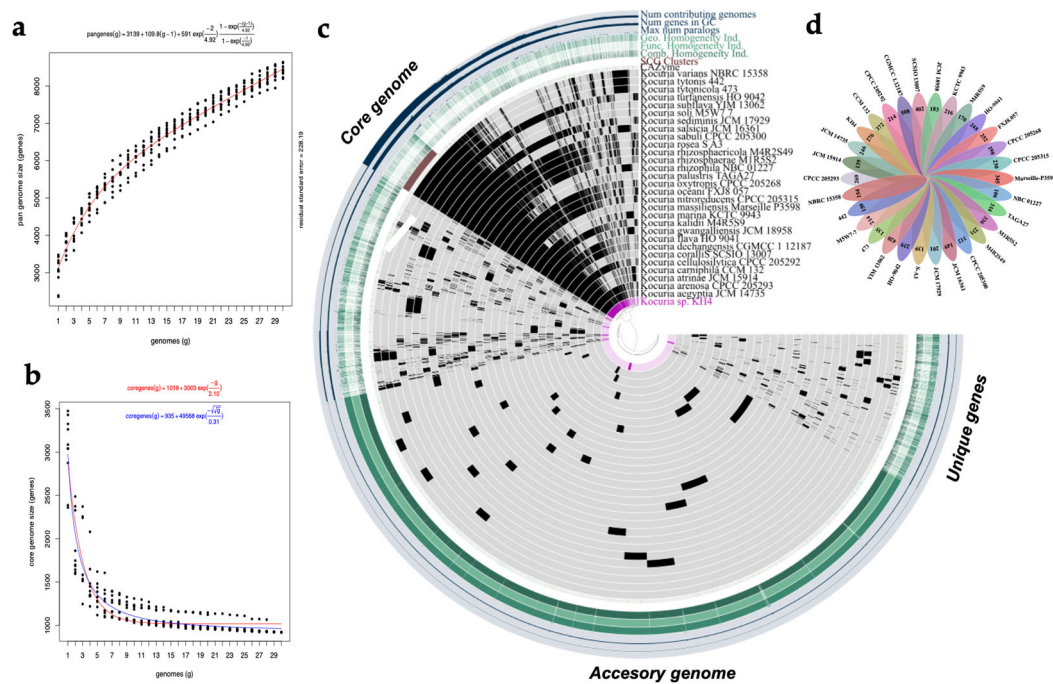


Figure 4. Analysis of the *Kocuria* genus pangenome. (a) Curve and estimation of pangenome size. (b) Curve and estimation of core genome size. (c) Distribution of core genome, accessory genome, and unique genes across the *Kocuria* pangenome in anvio v8. (d) Number of unique genes by strain.

3.4. Metal Resistance Genes in *Kocuria* sp. KH4

The genetic profile of metal resistance in *Kocuria* sp. KH4 was inferred from the information encoded in its genome by comparing it with the MetHMMDB database, which includes 254 hidden Markov models (HMMs) covering 121 microbial genes associated with metal resistance (MMRGs) and their functions. The identified genes were grouped according to the type of metal to which they confer resistance and the functional mechanism involved (Figure 5a). A predominance of genes associated with homeostasis and resistance to essential metals such as iron (37 genes), nickel (31), and zinc (20) was observed, followed by copper (9) and toxic metals such as mercury and arsenic (4 genes each). In terms of molecular mechanisms, the most represented correspond to ABC transporters (47 genes) and other nonspecific resistance mechanisms (46 genes), followed by metal-binding proteins (8), biotransformation enzymes (6), and P-type ATPases (4). In detail, genes related to iron transport and uptake were detected, such as *fecD*, *fecE*, *fbpB*, and *fbpC*, which are involved in the acquisition of this essential element under limiting conditions. Likewise, genes linked to nickel absorption and transport were identified, including *nikB*, *nikC*, *nikD*, and *nikE*, which encode permeases and importers of the Nik system, essential for the uptake and intracellular transport of the cation. In the case of zinc, genes from the ZnuABC system and the *troB* and *zupT* transporters were found, which participate in both the entry and intracellular regulation of Zn^{2+} . Determinants for copper, mainly Cu-transporting ATPases (copA-like), and for cadmium, cobalt, and zinc via the *czcD* efflux system were also detected. Furthermore, genes related to resistance to toxic metals were observed, including *aioA* and *acr3* for arsenic, as well as several copies of the *merA* gene, which encodes mercuric reductases involved in mercury detoxification. Finally, genes with nonspecific but potentially relevant functions in metal resistance were identified, such as *mntH* (divalent cation transporter), *actP* (cation symporter), and the *corC* system, associated with magnesium and cobalt homeostasis (Table S2).

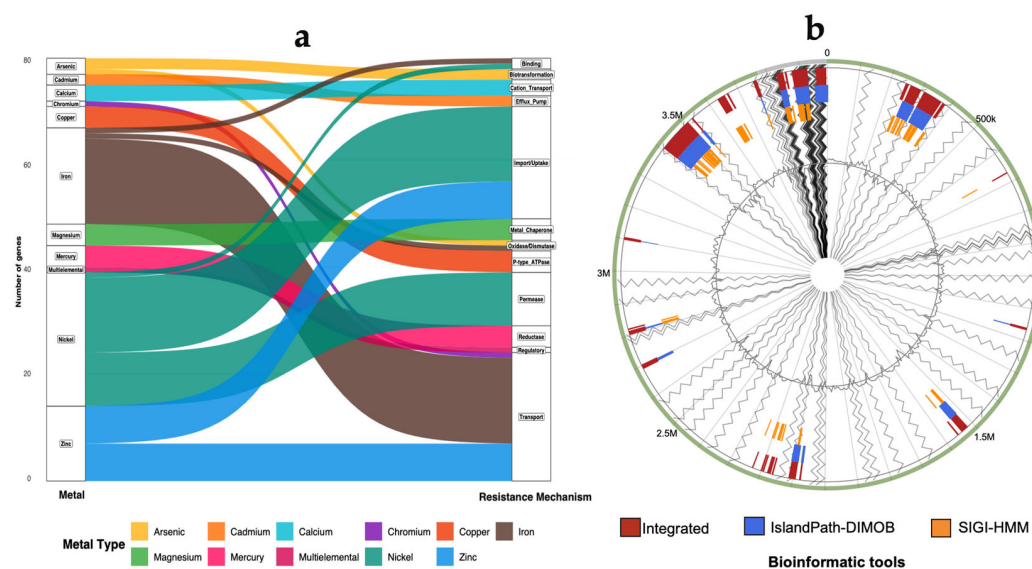


Figure 5. Distribution of genes related to metal resistance in *Kocuria* sp. KH4. (a) Alluvial diagram showing metal-resistance genes grouped by element and by their associated biochemical mechanisms. (b) Distribution and size of the genomic islands identified by three bioinformatic algorithms.

3.5. Genomic Islands (GIs) in *Kocuria* sp. KH4

A comprehensive genomic analysis aimed at identifying unusual patterns in nucleotide composition revealed 48 genomic islands (GIs) in *Kocuria* sp. KH4. Table S3 and Figure 5b show the start and end coordinates of the GIs, their sizes (7.7–62.7 kb), and the products they encode. These islands contain numerous transposases (IS3, IS4, IS5, and IS402 families), integrases/recombinases (including serine- and tyrosine-recombinases and resolvases/invertases), a relaxase protein of the MOB-F family, and components of conjugation systems (such as TraD/TraG and TrbL/VirB6). These features suggest that these segments are mobile genetic elements (MGEs). Additionally, multiple transcriptional regulators belonging to families such as MerR, ArsR/SmtB, MarR, IclR, and XRE were detected, as well as two-component systems, particularly those associated with sensing metal toxicity and oxidative stress. Regarding metal tolerance, some GIs harbor genes that encode cation diffusion facilitators (CDFs), efflux pumps, and copper-resistance proteins (CopD). These genes also encode domains associated with metal sequestration and detoxification.

3.6. Biosynthetic Gene Clusters (BGCs) in *Kocuria* spp.

For the purpose of predicting the biotechnological potential associated with the production of specialized metabolites, genome mining was performed to detect BGCs in the *Kocuria* sp. KH4 strain and in the 30 reference strains used for phylogenomic analyses. The results revealed that the genome of *Kocuria* sp. KH4 harbors six classes of BGCs, which encode genes associated with the synthesis of NAPAA (non- α -poly-amino acids), NRPS (nonribosomal peptide synthetases), T3PKS (type III polyketide synthases), terpenes, and Ni-siderophores (Figure 6). These BGCs are shared with other species, as indicated in the absence and presence matrix in Figure S1. On a global scale, 192 BGCs were identified among the *Kocuria* species analyzed, corresponding mainly to the NRPS, RiPPs (ribosomally synthesized and post-translationally modified peptides), PKS (polyketide synthases), and terpenes (Table 1). At the interspecific level, it was observed that certain classes of BGCs are shared by most species, suggesting the conservation of essential biosynthetic pathways. For instance, terpene and NRPS-like clusters were detected in almost all analyzed genomes. In contrast, PKS-type BGCs (particularly T3PKS) and those associated with siderophores showed a more restricted distribution, predominating in species such

as *K. marina*, *K. sediminis*, and *K. flava*. Likewise, species such as *K. rosea*, *K. polaris*, and *K. palustris* stood out for presenting complex combinations of hybrid BGCs, which integrate NRPS–PKS or RiPP–terpene modules, reflecting greater metabolic plasticity. In contrast, species such as *K. rhizophila* and *K. kristinae* showed a more limited repertoire, dominated by simple terpenes and RiPPs. Overall, the analysis suggests that, although the genus *Kocuria* retains a common core of BGCs, there is notable variability in the number and type of clusters between species, reflecting their metabolic diversification and adaptation.

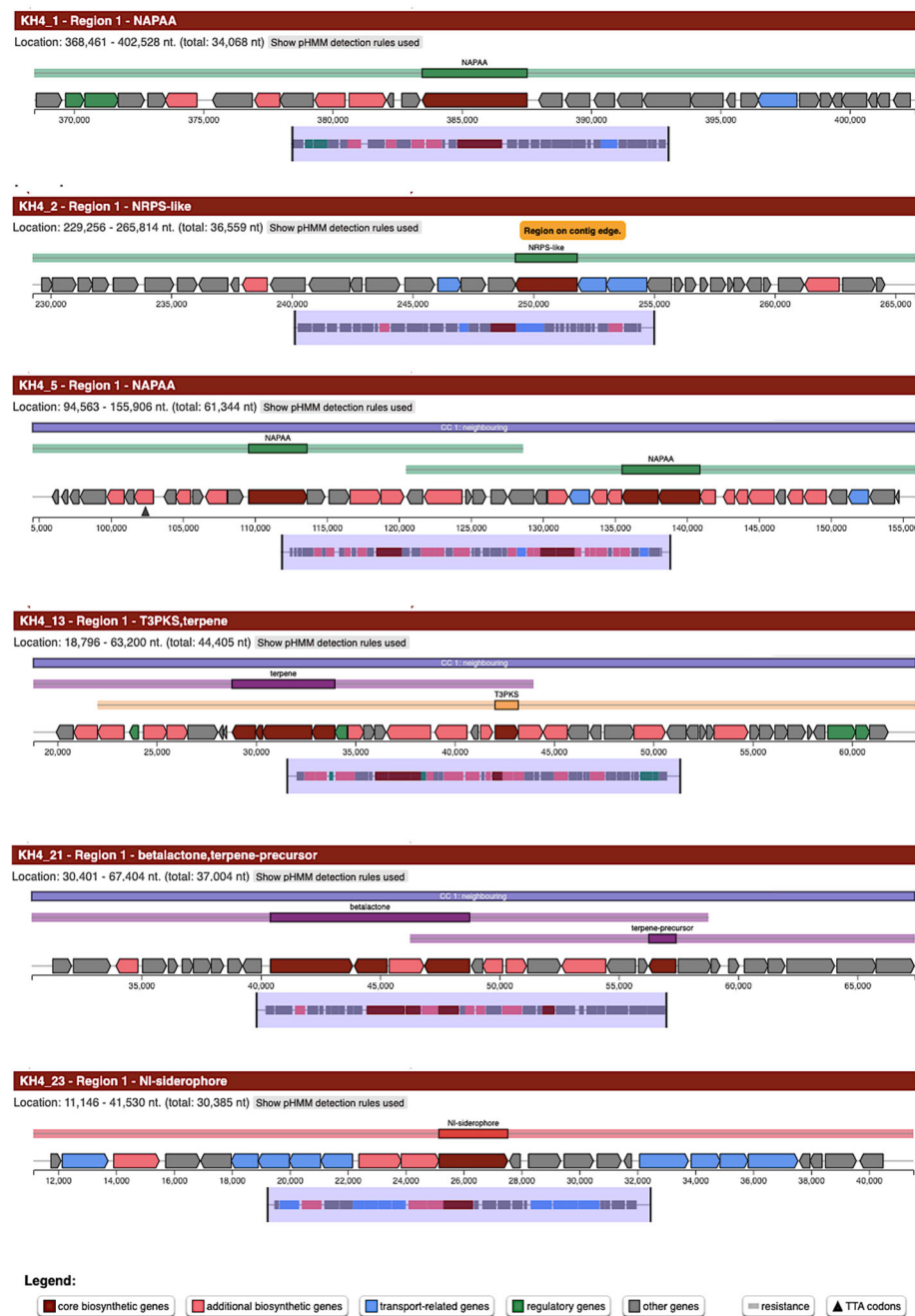


Figure 6. Biosynthetic gene clusters (BGCs) in *Kocuria* sp. strain KH4. The location, genetic arrangement, and class of BGCs are shown. NAPAA (non- α -poly-amino acids), NRPS (nonribosomal peptide synthetases), T3PKS (type III polyketide synthases), terpenes, and Ni-siderophores. The colors of the boxes denote the function of proteins encoded by each gene in the biosynthesis of specialized metabolites.

Table 1. Classes of biosynthetic gene clusters (BGCs) identified in genomes of *Kocuria* species.

Species	BGC Class
<i>Kocuria</i> sp. KH4	NAPAA; NI-siderophore; NRPS-like; T3PKS.terpene; terpene-precursor.betalactone
<i>Kocuria aegyptia</i> JCM 14735	NAPAA; NI-siderophore; NRPS-like; RiPP-like; T3PKS.terpene; terpene; terpene-precursor.betalactone
<i>Kocuria arenosa</i> CPCC 205293	NAPAA; NRPS-like; RiPP-like; T3PKS.terpene; terpene; terpene-precursor.betalactone
<i>Kocuria atrinae</i> JCM 15914	NAPAA; RiPP-like; T3PKS; terpene; terpene-precursor; terpene-precursor.betalactone
<i>Kocuria carniphila</i> CCM 132	NAPAA; NI-siderophore; T3PKS; terpene; terpene-precursor; terpene-precursor.betalactone
<i>Kocuria cellulosilytica</i> CPCC 205292	NAPAA; NRPS-like; T3PKS.terpene; terpene-precursor.betalactone
<i>Kocuria coralli</i> SCSIO 13007	NI-siderophore; T3PKS; betalactone; terpene; terpene-precursor
<i>Kocuria dechangensis</i> CGMCC 1.12187	NAPAA; NI-siderophore; NRPS-like; RiPP-like; T3PKS.terpene; terpene-precursor.betalactone
<i>Kocuria flava</i> HO-9041 1	NAPAA; NI-siderophore; NRPS-like; RiPP-like; T3PKS.terpene; azole-containing-RiPP; terpene-precursor.betalactone
<i>Kocuria gwangalliensis</i> JCM 18958	NAPAA; RiPP-like; T3PKS; terpene; terpene-precursor; terpene-precursor.betalactone
<i>Kocuria kalidii</i> M4R5S9	NAPAA; NI-siderophore; NRPS-like; RiPP-like; T3PKS.terpene; terpene-precursor.betalactone
<i>Kocuria marina</i> KCTC 9943	NAPAA; NI-siderophore; T3PKS; terpene; terpene-precursor.betalactone
<i>Kocuria massiliensis</i> Marseille-P3598 2	NI-siderophore; RiPP-like; terpene-precursor.betalactone
<i>Kocuria nitroreducens</i> CPCC 205315	NAPAA; NRPS-like; RiPP-like; T3PKS.terpene; terpene-precursor.betalactone
<i>Kocuria oceani</i> FXJ8.057 23	NAPAA; NI-siderophore; NRPS-like; T3PKS.terpene; terpene-precursor.betalactone
<i>Kocuria oxytropis</i> CPCC 205268	NAPAA; NRPS-like; betalactone; linaridin; T3PKS.terpene; terpene; terpene-precursor.betalactone
<i>Kocuria palustris</i> TAGA27	NI-siderophore; terpene; terpene-precursor
<i>Kocuria rhizophila</i> NBC 01227	NAPAA; NI-siderophore; RiPP-like; T3PKS; terpene; terpene-precursor.betalactone
<i>Kocuria rhizosphaerae</i> M1R5S2	NAPAA; NI-siderophore; NRPS-like; T3PKS.terpene; terpene-precursor.betalactone
<i>Kocuria rhizosphaericola</i> M4R2S49	NAPAA; NRPS-like; betalactone; T3PKS.terpene; terpene-precursor
<i>Kocuria rosea</i> S-A3	NAPAA; NI-siderophore; NRPS-like; RiPP-like; T3PKS.terpene; terpene; terpene-precursor.betalactone
<i>Kocuria sabuli</i> CPCC 205300	NAPAA; NI-siderophore; NRPS-like; RiPP-like; T3PKS; T3PKS.terpene; indole; terpene-precursor.betalactone
<i>Kocuria salsicia</i> JCM 16361	NAPAA; NRPS.NRP-metallophore; RiPP-like; T3PKS; terpene; terpene-precursor.betalactone
<i>Kocuria sediminis</i> JCM 17929	NAPAA; NI-siderophore; NRPS-like; T3PKS.terpene; terpene; terpene-precursor.betalactone

Table 1. Cont.

Species	BGC Class
<i>Kocuria soli</i> M5W7-7	NAPAA; NI-siderophore; hglE-KS; terpene; terpene-precursor
<i>Kocuria subflava</i> YIM 13062	NAPAA; terpene; terpene-precursor
<i>Kocuria turfanensis</i> HO-9042	NAPAA; NI-siderophore; NRPS-like; T3PKS.terpene; terpene-precursor.betalactone
<i>Kocuria tytonicola</i> 473	NAPAA; NI-siderophore; T3PKS; terpene; terpene-precursor.betalactone
<i>Kocuria tytonis</i> 442	NAPAA; NI-siderophore; RiPP-like; T3PKS; terpene; terpene-precursor.betalactone
<i>Kocuria varians</i> NBRC 15358	NAPAA; NRPS.NRP-metallophore; RiPP-like; terpene; terpene-precursor.betalactone

NAPAA (non- α -poly-amino acids), NRPS (nonribosomal peptide synthetases), T3PKS (type III polyketide synthases), RiPPs (ribosomally synthesized and post-translationally modified peptides).

4. Discussion

The genus *Kocuria* includes widely distributed actinobacteria found in soils, deserts, marine environments, rhizospheres, and in association with plant tissues. From a biotechnological point of view, it is notable for its production of carotenoids, antibiotics, and exopolysaccharides, as well as its potential in agriculture and environmental remediation. In the present study, *Kocuria* sp. strain KH4 was isolated from sulfide bearing tailings (“La Concha”) in Guerrero, Mexico. A functional analysis of its genome was performed to determine its precise taxonomic classification, identify genes associated with metal tolerance or resistance, and explore its genotypic potential for the production of secondary or specialized metabolites.

Various strains of the genus *Kocuria* have demonstrated an arsenal of metabolic strategies necessary to colonize environments with extreme geochemical characteristics and oligotrophic conditions. For example, *K. arsenatis* sp. nov., resistant to arsenic, was isolated as an endophyte of *Prosopis laevigata* growing on mining tailings in San Luis Potosí (Mexico) [9]. Similarly, *Kocuria* sp. CRB15, a strain highly resistant to Cu, was obtained from the rhizosphere of *Saccharum spontaneum* in copper mines in Rakha [40]. Likewise, *K. flava* CR1 has been recovered from copper-contaminated soils in Urumqi, China [21], while *K. dechangensis* RSKoc01 was found associated with Trifolium roots in lead-zinc mines [41]. Taken together, these findings show that *Kocuria* not only tolerates but thrives in metal-rich niches, highlighting its ecological relevance and biotechnological potential for bioremediation and phytoremediation of contaminated environments.

Genomic analysis of *Kocuria* sp. KH4 revealed a set of features consistent with physiological and metabolic adaptation to metalliferous and stressful habitats. Its genome size (~3.89 Mb), high GC content (73.2%), and proportion of genes with functional annotation according to COG (~78%) are within the known range for the genus. *Kocuria* species have genome sizes between 2.7 and 4.0 Mb, with an approximate average of 3.7 Mb and a GC content of 60–75%, an attribute associated with greater structural stability and resistance to environmental stress [42]. Phylogenomic analyses placed strain KH4 in a monophyletic clade separate from the rest of the species, and it showed no close relationship (ANI < 95%) with reference strains deposited in the GTDB (Genome Taxonomy Database; release 19 October 2024). These results, complemented by Genome Relatedness Indices (OGRIIs), suggest that this bacterium represents a new genomospecies. A genomospecies is a bacterial taxonomic group defined exclusively on the basis of its genomic relationship, i.e., by the degree of overall similarity between its complete genomes. Unlike novel species

traditionally defined by phenotypic, physiological, or ecological characteristics, a genomospecies does not require or consider phenotypic traits for its delimitation [43]. In agreement, recent studies have demonstrated the usefulness of phylogenomic analyses, ANI, AAI, and dDDH to describe new lineages within the genus *Kocuria* [44]. However, culture-dependent studies—such as the use of substrates, growth under variable conditions (temperature, pH, salinity, etc.), and fatty acid profile analysis—are still required for the formal description of novel bacterial species [45].

In functional terms, *Kocuria* sp. KH4 exhibits a clear bias toward genes involved in amino acid and carbohydrate metabolism, as well as in the transport and metabolism of inorganic ions. The latter category is particularly relevant, as it coincides with the selective pressures associated with the “La Concha” sulfide bearing tailings. These are characterized by high concentrations of metals [As (1140–11,800 mg kg^{−1}), Cd (8–780 mg kg^{−1}), Cu (71.8–1320 mg kg^{−1}), Fe (6000–12,300 mg kg^{−1}), Pb (10,100–43,700 mg kg^{−1}), Zn (780–10,000 mg kg^{−1})] in highly bioavailable chemical forms, a wide diversity of sulfide minerals, and limited nutrient availability [28]. These abiotic factors act as ecological filters that shape the structure and functionality of microbial communities, giving them unique functional adaptive traits [46]. In this context, the genome of *Kocuria* sp. KH4 showed a broad repertoire of genes associated with metal resistance, including those associated with iron, nickel, zinc, copper, arsenic, and mercury. These genes encode ABC transporters, P-type ATPases, metal-binding proteins, and biotransformation enzymes. These genes encode proteins and enzymes that together orchestrate mechanisms of metal tolerance such as biotransformation, bioadsorption, bioprecipitation, and bioaccumulation, among others.

The biotransformation is one of the most important mechanisms, as it allows the redox state of metals to be modified, reducing their mobility and toxicity [47]. In several species of *Kocuria* spp. isolated from metal-rich environments—for example, *K. arsenatis* and *K. palustris*—enzymes such as arsenate reductase have been described, capable of converting As (V) to As (III), which is subsequently expelled by Acr3-type transporters [9,48]. Similarly, several actinobacteria, including *Kocuria* spp., possess *merA*, which reduces Hg (II) to Hg (0) [44], a mechanism also present in the KH4 strain. Besides biotransformation, *Kocuria* spp. employ mechanisms such as biosorption, where metal ions passively adhere to the cell surface through electrostatic interactions with functional groups such as phosphate, carboxylate, or sulfate present in the cell wall or exopolysaccharides (EPS) [49]. This process has been demonstrated in *K. rosea*, used in the removal of U(VI) and Hg [50,51]. Another important mechanism is bioprecipitation, whereby bacteria transform highly soluble metals into thermodynamically stable, low-solubility chemical forms such as carbonates, sulfides, or oxides [52]. Microbial-induced carbonate precipitation (MICP), facilitated by urease activity, has been documented in *K. flava* CR1, which is capable of precipitating copper through the formation of metal carbonates [21]. Another strategy is bioaccumulation, which allows for the active incorporation of metals into the cell, where they are sequestered by specific metalloproteins and chaperones [53]. This strategy, widely distributed in Actinobacteria, has also been reported in species such as *K. rhizophila* 14ASP and *Kocuria* sp. BR1-36 [54,55].

Forty-eight genomic islands were identified in the genome of *Kocuria* sp. KH4, ranging in size from 7.7 kb to 62.7 kb and collectively encoding a wide variety of functions and mobile genetic elements (MGEs). Similarly, genomic studies in *K. rhizophila* and *K. pseudorhizophila* close species have reported the presence of new insertion sequences (ISs) (ISKrh4–7, ISKrh1–3, and IS481) and specific mobile elements [56], which is consistent with the recurrence of transposases, integrases, and recombinases, as well as a relaxase and transfer proteins (MobF, TraD/TraG, TrbL/VirB6) in our strain. This set of MGEs has been involved

in the horizontal mobilization of genetic determinants between strains, favoring their evolution and adaptation to stressful environments [57].

One of the aspects where the genetic diversity of the *Kocuria* genus is most evident is in the presence of secondary metabolite biosynthesis clusters (BGCs). Multiple BGCs were identified in the genome of *Kocuria* sp. KH4, including NRPS (nonribosomal peptide synthetases), T3PKS (type III polyketide synthases), NAPAA (non- α -poly-amino acids), terpenes, and siderophores. In other microorganisms, these compounds are associated with the production of antibiotics, pigments, antioxidant agents, and molecules involved in metal mobilization or complexation [58,59]. NRPS are multi-modular megaenzymes that synthesize nonribosomal peptides from proteinogenic and non-proteinogenic amino acids [60]. In contrast, T3PKS are monofunctional enzymes that produce polyketides derived from acetone-malonate units [61]. NAPAA represent polymers whose amino acids are linked through alternative positions (β , γ , or δ) and are usually synthesized through specialized pathways that modify amino acids through transamidation, oxidation-reduction, or cyclization reactions [62]. Bacterial terpenes, derived from IPP and DMAPP, include monoterpenes, sesquiterpenes, diterpenes, and carotenoids [63]. Siderophores, on the other hand, are low-molecular-weight molecules with high affinity for iron and, in some cases, other metals, and they can be synthesized via NRPS-dependent or NRPS-independent pathways [64].

The chemical nature and biological activity of the metabolites produced by *Kocuria* have been widely reported in *Kocuria* isolates, although the BGCs involved are not known in all cases. For example, *K. sediminis* As04 produces specific carotenoids (phytoene and β -cryptoxanthin) with potential industrial applications [65], while *K. flava* NIO_01 harbors multiple BGCs corresponding to terpenes, T3PKS, and siderophores, as well as NRPS and NAPAA [18]. A notable example is *K. palustris*, which produces the antibiotic kocurin, a thiazolyl peptide with activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) [66]. Further notable cases are *Kocuria* strains that have the machinery for the biosynthesis of alkanoyl imidazoles with antimicrobial activity (nocarimidazoles) [67]. Furthermore, several novel species, including *K. rhizosphaerae* sp. nov., *K. kalidii* sp. nov., and *K. rhizosphaericola* sp. nov., are capable of degrading phenolic acids [44]. Also, multiple strains of *Kocuria* spp. have been shown to produce indole-3-acetic acid (IAA), giving them the ability to stimulate plant growth [1].

The metabolic versatility and genomic plasticity of *Kocuria* sp. KH4 involved in the biosynthesis of multiple secondary or specialized metabolites, as well as their intrinsic resistance to diverse toxic metals, suggest a functional link between both systems. Several microbial metabolites can act as protective molecules against metal stress, either as antioxidant agents that counteract the generation of reactive oxygen species (ROS) derived from redox-active metals, or as siderophores that participate in iron acquisition and heavy metal chelation. This type of co-adaptation has been reported in several metalliferous environments [68–71] and could explain, at least partially, the ecological success of actinobacteria in mine tailings.

5. Conclusions

The genomic analysis of *Kocuria* sp. KH4 revealed a novel genomospecies with remarkable metabolic versatility and adaptive potential. The presence of multiple metal resistance genes and six biosynthetic gene clusters (BGCs) for nonribosomal peptides, polyketides, terpenes, bacteriocins, and siderophores highlights its capacity to produce diverse secondary metabolites. These features suggest an interplay between metal tolerance and secondary metabolism that confers ecological resilience in metal-rich environments. Overall, *Kocuria* sp. KH4 represents a promising source of bioactive compounds with

potential applications in environmental and industrial biotechnology. Further functional studies and complementary Omics approaches (including transcriptomics, proteomics, and metabolomics) will be essential to fully uncover its biosynthetic potential and to characterize its biological activities.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microbiolres16120255/s1>. Table S1. *Kocuria* genomes used for phylogenetic analysis in this study; Table S2. Unique genes in *Kocuria* sp. KH4 compared to other *Kocuria* species; Table S3. Data from genomic islands (GIs) in *Kocuria* sp. KH4; Figure S1: Presence/Absence matrix of biosynthetic gene clusters (BGCs) identified in *Kocuria* species. NAPAA (non- α -poly-amino acids), NRPS (nonribosomal peptide synthetases), T3PKS (type III polyketide synthases), RiPPs (ribosomally synthesized and post-translationally modified peptides).

Author Contributions: Conceptualization, J.L.A.-N.; Methodology, G.C.-F. and J.L.A.-N.; Software, G.C.-F.; Validation, G.C.-F.; Formal analysis, G.C.-F., A.A.-M. and J.R.-G.; Investigation, L.X.Z.-M. and J.R.-G.; Resources, J.L.A.-N.; Data curation, G.C.-F.; Writing—original draft, G.C.-F., L.J.G.-G., A.A.-M., L.X.Z.-M., J.R.-G. and J.L.A.-N.; Writing—review and editing, L.J.G.-G., A.A.-M., L.X.Z.-M., J.R.-G. and J.L.A.-N.; Visualization, L.J.G.-G.; Supervision, J.R.-G. and J.L.A.-N.; Project administration, J.R.-G. and J.L.A.-N.; Funding acquisition, J.L.A.-N. All authors have read and agreed to the published version of the manuscript.

Funding: Genome sequencing was funded by the GetGenome initiative at the Sainsbury Laboratory in Norwich, UK, with support from the Gatsby Charitable Foundation and the Biotechnology and Biological Sciences Research Council, as part of the “GetGenome Mexico 2023” project. Grant number: ID5706.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original data presented in the study are openly available in NCBI BioProject PRJNA1365121 (<https://www.ncbi.nlm.nih.gov/bioproject/?term=+PRJNA1365121>), accessed on 29 November 2025.

Acknowledgments: We thank UATI for their expert technical support and server maintenance at Centro de Ciencias Genómicas, UNAM.

Conflicts of Interest: The authors declare no conflicts of interest.

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