Comparative genomics analysis of *Chryseobacterium* sp. KMC2 reveals metabolic pathways involved in keratinous utilization and natural product biosynthesis

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Abstract

Several efforts have been made to valorize keratinous materials, an abundant and renewable resource. Despite these attempts to valorize products generated from keratin hydrolysate, either *via* chemical or microbial conversion, they generally remain with an overall low value. In this study, a promising keratinolytic strain from the genus *Chryseobacterium* (*Chryseobacterium* sp. KMC2) was investigated using comparative genomic tools against publicly available reference genomes to reveal the metabolic potential for biosynthesis of valuable secondary metabolites. Genome and metabolic features of four species were compared, shows different gene numbers but similar functional categories. We successfully mined eleven different secondary metabolite gene clusters of interest from the four genomes, including five common ones shared across all genomes. Among the common metabolites, we identified gene clusters involved in biosynthesis of flexirubin-type pigment, microviridin, and siderophore, all showing remarkable conservation across the four genomes. Unique secondary metabolite gene clusters were also discovered, for example, ladderane from *Chryseobacterium* sp. KMC2. Additionally, this study provides a more comprehensive understanding of the potential metabolic pathways of keratin utilization in *Chryseobacterium* sp. KMC2, with the involvement of amino acid metabolism, TCA cycle, glycolysis/gluconeogenesis, propanoate metabolism, and sulfate reduction. This work uncovers the biosynthesis of secondary metabolite gene clusters from four keratinolytic *Chryseobacterium* spp. and shades lights on the keratinolytic potential of *Chryseobacterium* sp. KMC2 from a genome-mining perspective, providing alternatives to valorize keratinous materials into high-value natural products.

47 **Importance**

48 Keratin is an abundant and renewable resource from slaughterhouses or the poultry industry.
49 Low-value products such as animal feed and fertilizer were generated from these feedstocks
50 based on conventional processing like chemical conversion. In fact, microorganisms possess the
51 potential to synthesize valuable natural products. In this work, we explored the metabolic
52 potential of *Chryseobacterium* sp. KMC2, which was isolated with efficient keratinolytic
53 capacity from a previous study. Comparative genomics analysis displayed similar functional
54 categories against three publicly available reference genomes of keratin-
55 degrading *Chryseobacterium* spp.. Eleven different secondary metabolite gene clusters of interest
56 were mined among four genomes, including five common and unique ones. Furthermore, we
57 provide a more comprehensive understanding of metabolic pathways on keratin utilization
58 in *Chryseobacterium* sp. KMC2, with the involvement of amino acid assimilation and sulfate
59 reduction. These findings contribute to expanding the application of *Chryseobacterium* sp.
60 KMC2 on the valorization of keratinous materials.

61

62 **Keywords:** keratinous materials, metabolic potential, genome mining, gene clusters,
63 degradation pathways

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70 **Introduction**

71 Keratin is the most abundant proteins in epithelial cells, constituting the bulk of epidermal
72 appendages such as hair and feather (1, 2). Keratinous materials represent an abundant protein
73 source, particularly originating from the commercial slaughterhouses or poultry farms (3). They
74 contain peptides and amino acids, which are renewable natural resources with great potential in
75 sustainable development (4). However, keratin is an insoluble protein with highly cross-linked
76 disulfide bonds giving it a tough and recalcitrant structure (5). Many attempts have been made to
77 hydrolysis keratinous materials in terms of physicochemical treatment, enzymatic hydrolysis,
78 and microbial conversion (6, 7). The hydrolysis products of keratinous materials have been used
79 for animal feed (8) and fertilizer (6, 9) based on conventional processing.

80

81 Microorganisms represent one of the most important natural sources, which have the potential to
82 generate bioactive compounds such as antibiotics, biofuels, and natural pigments derived from
83 cellular metabolites (10, 11). For example, *Yarrowia lipolytica* has been used to convert different
84 renewable feedstocks to high-value metabolites (12). Similarly, *Escherichia coli* has become one
85 of the best cell reactors to produce alcohols, organic acids, biodiesel, even hydrogen by utilizing
86 renewable resources (13). Other bacteria such as *Bacillus subtilis* (14), *Caldicellulosiruptor*
87 *bescii* (15), *Corynebacterium glutamicum* (16), and *Ruminococcaceae* bacterium (17) were
88 identified and evaluated with the capacity to generate different products by converting renewable
89 carbon sources. Notably, some microorganisms were reported to degrade keratinous waste
90 effectively (18). Exploring keratinolytic potential of these microbes to generate high value-added
91 products is an important step to recycle and valorize keratinous materials.

92

93 Molecular mechanisms of microbial keratin degradation are still not fully understood, while
94 genome sequencing offers possibilities to reveal the metabolic potential behind efficient
95 microbial degradation (19). Novel keratinolytic enzymes were identified from the genome of
96 *Bacillus pumilus* 8A6, an efficient keratin degrader (20). Furthermore, going beyond the
97 degradation reaction itself, genomes can also be minded for valuable accessory functions of
98 interest, adding more values to the microbial conversion processes. For instance, gene clusters
99 and biosynthesis pathways of secondary metabolites could be disclosed from genomes *via*
100 adequate analysis tools (21, 22). A total of 104 putative biosynthetic gene clusters (BGCs) for
101 secondary metabolites were predicted from nine *Ktedonobacteria* genomes (23). Secondary
102 metabolites were identified and linked to gene clusters based on the comparison and mining of
103 six genomes belonging to diverse *Aspergillus* species, successfully fueling industrial
104 biotechnology initiatives and medical research (24). Therefore, using the genomes of
105 keratinolytic microbial species in a similar way would represent a promising approach to
106 discover biosynthetic gene clusters of secondary metabolites of interest, excavating the full
107 application potential of these microbes.
108
109 Recently, several studies based on different environments have revealed the remarkable potential
110 of representative taxa from the *Chryseobacterium* genus for keratin degradation using isolation,
111 activity tests and genome sequencing (25, 26). In this study, a novel strain *Chryseobacterium* sp.
112 KMC2 was previously obtained from an enrichment procedure, displaying a potent capacity of
113 keratin degradation (27). The genome of *Chryseobacterium* sp. KMC2 was analyzed and
114 compared with publicly available genomes of other keratinolytic *Chryseobacterium* spp. to
115 clarify the genomic basis of keratin degradation, and to unravel hidden biosynthetic gene clusters

116 of interest. Subsequently, the metabolic pathways associated with keratin degradation were
117 constructed, providing deeper insight into the yet obscure keratinolytic processes. This work
118 reveals the keratinolytic potential of *Chryseobacterium* spp. and mined potential accessory gene
119 clusters of secondary metabolites, which could i) contribute to optimizing the processes of
120 keratin degradation and ii) broaden the perspective to generate added-value products from
121 keratin hydrolysate.
122
123
124 **Results and discussion**
125
126 **Genome feature comparison of four keratinolytic *Chryseobacterium* strains**
127 *Chryseobacterium* sp. KMC2 originated from a river-bank soil sample, and displayed a potent
128 degradation ability toward milled pig bristle and hooves (27, 28). The genome of
129 *Chryseobacterium* sp. KMC2 was sequenced and compared to three reference genomes of
130 *Chryseobacterium* spp. Including: i) *Chryseobacterium camelliae* Dolsongi-HT, isolated from
131 green tea leaves (29); ii) *Chryseobacterium gallinarum* strain DSM 27622, isolated from chicken
132 (30); and iii) *Chryseobacterium* sp. strain P1-3 isolated from poultry waste (31), which all
133 display keratinolytic capacity (Table 1). *Chryseobacterium* sp. KMC2 showed distinct genome
134 feature from the other known keratinolytic strains. The genome size of *Chryseobacterium* sp.
135 KMC2 is 5.28 Mbp, larger than the other three genomes, which ranged from 4.38 Mbp to 4.63
136 Mbp. A total of 4,773 genes were predicted from *Chryseobacterium* sp. KMC2 genome, and
137 about 4,000 genes were annotated from the other three genomes. Besides, the GC content ranges
138 from 36.33% to 41.80% in *Chryseobacterium* spp. genomes. Furthermore, the whole-genome

139 phylogenetic tree was constructed with other eight publically available *Chryseobacterium* spp.
140 genomes (Fig. 1a), showing the highly similarity among *Chryseobacterium gallinarum* strain
141 DSM 27622 and *Chryseobacterium* sp. strain P1-3. Notably, *Chryseobacterium* sp. KMC2 and
142 *Chryseobacterium camelliae* Dolsongi-HT have closer evolutionary relationship with other
143 *Chryseobacterium* species. Although the keratinolytic capacity within these eight strains is still
144 not clear, more keratinolytic species are expected to be found in this genus according to the
145 phylogenic tree analysis. Four strains among this genus have documented with actual keratin
146 degradation capability (29-31), while *Chryseobacterium* sp. KMC2 may possess different
147 keratinolytic potential encoded in its larger genome compared to three other *Chryseobacterium*
148 strains.
149
150 **Metabolic potential comparison of four keratinolytic *Chryseobacterium* genomes**
151 About 40% of the genes from the four genomes were classified into various functional categories
152 based on the KEGG database. The vast majority of annotated genes belonged to metabolism,
153 genetic information processing, environmental information processing, and cellular processes
154 (Fig. 1b). The functional categories of the genomes were overall highly similar, with ~85% of
155 annotated genes assigned to "metabolism" (category A) which included ~1.000 genes into the
156 sub-category "global and overview maps". Additionally, about 8% and 4% annotated genes from
157 each genome were assigned to "genetic information processing" (category B) and
158 "environmental information processing" (category C), respectively. The remaining annotated
159 genes belonged to "cellular processes" (category D), which occupied 3% of the annotated
160 genomes approximately.
161

162 Remarkably, each genome had more than 200 genes assigned into the "amino acid metabolism"
163 sub-category. Keratin is mainly composed of amino acids (3), which is ultimately the operational
164 carbon nutrient source exploited for microbial growth. Numerous amino acid metabolism-related
165 enzymes were annotated, revealing the genetic potential of these *Chryseobacterium* strains for
166 using keratin materials as energy sources. Of particular interest, several biosynthesis genes of
167 secondary metabolites were detected from the genomes, of which more than 20 genes were
168 annotated as “metabolism of terpenoids and polyketides” and around 40 genes were annotated as
169 “biosynthesis of other secondary metabolites” sub-category (Fig. 1b). Terpenoids are a group of
170 natural products with diverse commercial applications, which have been produced from
171 microbial cell factories (32). Many polyketides are considered as significant natural products
172 with broad applications in the agriculture and pharmaceutical industry (33). The metabolic
173 pathways related to polyketides biosynthesis production are well understood in some
174 microorganisms like *Streptomyces* which play a crucial role in industrial bioproduction (34).
175 This result indicates that these *Chryseobacterium* strains could have the potential to synthesize
176 high-value secondary metabolites such as terpenoids and polyketides from keratinous materials.
177

178 **Mining and comparing secondary metabolite gene clusters**

179 Genome mining is an effective approach to discover new natural products from microorganisms
180 based on “signature genes” detection or searching for specific patterns in gene sequences (35).
181 To explore the potential of producing high value chemicals from these four *Chryseobacterium*,
182 secondary metabolite gene clusters were predicted by using antiSMASH 5.0 mining pipeline (Fig.
183 2). In total, eleven different secondary metabolite gene clusters were identified.
184 *Chryseobacterium* sp. KMC2 possesses the largest number (15), while *Chryseobacterium*

185 *camelliae* Dolsongi-HT1 has the fewest (8). Ten gene clusters were predicted from the other two
186 strains. Five clusters are present in the four genomes, which are flexirubin-type pigment
187 (resorcinol and arylpolyene), microviridin, lanthipeptide, NRPS-like, and siderophore.
188 Remarkably, the flexirubin-type pigment is a typical metabolite produced from *Flavobacterium*
189 (36). Several species from *Chryseobacterium* were previously designated and known
190 as *Flavobacterium* owes to similar characteristics including the presence of yellow pigments (37).
191 Flexirubin-type pigment was isolated and characterized from *Chryseobacterium* sp. UTM-3T
192 (38). In addition, *Chryseobacterium* sp. KMC2 owns a unique gene cluster to produce ladderane.
193 Another unique natural product is beta-lactone from *Chryseobacterium camelliae* Dolsongi-HT1.
194 Ladderanes are hydrocarbon chains which were regarded as membrane lipid components
195 produced by anammox (anaerobic ammonia-oxidizing) bacteria uniquely, but the production is
196 not affordable due to their extremely low growth (39, 40). These results demonstrate that various
197 secondary metabolite gene clusters including both expected and unusual candidates were
198 discovered from *Chryseobacterium* genomes, which could turn into novel natural product
199 sources.
200
201 **Synteny analysis and features of secondary metabolite gene clusters**
202 Comparative genomics can reveal unique cluster and distribution patterns of secondary
203 metabolites in species (41). Five secondary metabolite gene clusters, predicted to be present in
204 the four genomes, were selected to explore the evolutionary relationship among four
205 *Chryseobacterium* strains. Three of them including flexirubin-type pigment, microviridin, and
206 siderophore display a conserved gene cluster structure from synteny analysis (Fig. 3, 4, and 5),
207 while the other two showed no evident synteny relation (Fig. S1 and S2).

208

209 **Flexirubin-type pigment.** Natural pigments have increasing applications in food,
210 pharmaceutical, and textile industries, owing to their advantages such as non-toxic,
211 biodegradable, and low allergenic potential compared to synthetic pigments (42). In particular,
212 flexirubin-type pigment has a potential antimicrobial and anti-tumoral activities (43).
213 Biosynthesis gene clusters of flexirubin-type pigment are conserved across the four tested
214 genomes, especially within *Chryseobacterium gallinarum* strain DSM 27622 and
215 *Chryseobacterium* sp. strain P1-3 (Fig. 3a). A total of 61 biosynthesis-related genes of
216 flexirubin-type pigment were predicted from *Chryseobacterium* sp. KMC2, including four core
217 biosynthesis genes. One of the core biosynthesis genes was annotated as 3-oxoacyl-(acyl carrier
218 protein) synthase III (*Flex11*), and the other three were annotated as Beta-ketoacyl synthases
219 (*Flex21*, *Flex24*, and *Flex40*). Besides, transport-related genes and regulatory genes were
220 predicted from the gene cluster. A previous study identified the molecular structure of flexirubin-
221 type pigment isolated from a *Chryseobacterium* sp. UTM-3T (38). According to the products
222 from core biosynthesis genes and their molecular structures, a proposition of biosynthesis
223 pathway was established (Fig. 3b), where flexirubin-type pigment is generated from resorcinol
224 and arylpolyene. Further transcriptomics and metabolomics analysis would be required to
225 confirm the validity of this potential pathway discovery.

226

227 **Microviridin.** Microviridins represent a group of ribosomally synthesized peptides under post-
228 translational modifications, which have been mainly isolated from cyanobacteria and present
229 potent serine-type protease inhibitory activities (44, 45). These properties could make
230 microviridin serve as the natural antimicrobial agents for developing potential drugs.

231 Biosynthesis gene clusters of microviridin from four *Chryseobacterium* genomes show a highly
232 conserved structure with a similarity greater than 71% from most gene synteny analysis (Fig. 4a).
233 There are 23 biosynthesis genes of microviridin that were predicted from *Chryseobacterium* sp.
234 KMC2. Two core biosynthetic genes (A and B) were identified from genomes and transport-
235 related genes were also been discovered. Besides, amino acid sequences of mvdA and mvdB
236 were aligned, showing that multiple motifs from mvdA and mvdB are conserved (Fig. 4bc).
237 Interestingly, many keratinases were reported to be classified as serine proteases, acting on the
238 molecular structure of keratin (46). This suggests that microviridins may regulate keratinolytic
239 activity, further characterizing and manipulating the microviridin synthetic pathway could
240 contribute to improving the keratin degradation efficiency.
241
242 **Siderophore.** Siderophores are ferric ion-specific chelators to scavenge iron from the
243 extracellular environment, which play important roles in virulence and oxidative stress tolerance
244 in microorganisms (47). It has been designed as a Trojan horse antibiotic to enter and kill
245 pathogenic bacteria (48), and also has shown the potential to decrease the growth of cancerous
246 cells (49). Biosynthesis gene cluster of siderophore shows a high synteny conservation among
247 *Chryseobacterium* sp. KMC2 and *Chryseobacterium camelliae* Dolsongi-HT1,
248 *Chryseobacterium gallinarum* strain DSM 27622 and *Chryseobacterium* sp. Strain P1-3,
249 respectively (Fig. 5). A total of ten genes were predicted from siderophore biosynthesis cluster of
250 *Chryseobacterium* sp. KMC2, and eight genes from the other three *Chryseobacterium* strains
251 separately. Functional description of each gene related to siderophore biosynthesis in
252 *Chryseobacterium* sp. KMC2 shows two core biosynthesis genes, and includes one regulatory
253 gene and one transport-related gene. This further suggests that those siderophore are potentially

254 fully functional molecular features that can be regulated on-demand and exported outside the cell
255 when needed.
256
257 **Metabolic pathways of keratin utilization in *Chryseobacterium* sp. KMC2 genome**
258 The main metabolic pathways related to keratin utilization in *Chryseobacterium* sp. KMC2
259 genome were investigated. These pathways included amino acid metabolism, TCA cycle,
260 glycolysis/gluconeogenesis, propanoate metabolism, and sulfate reduction (Fig. 6). A previous
261 study suggested that abundant amino acids are released during microbial degradation and used as
262 nutrient sources, such as leucine and aspartate (28). The metabolic pathways of amino acid
263 utilization were mapped from the genome of *Chryseobacterium* sp. KMC2. Most of the amino
264 acids are converted into intermediates of the TCA cycle. For instance, arginine can be converted
265 to succinate, then enter to TCA cycle after a multiple-steps enzyme reaction. Aspartate, tyrosine,
266 phenylalanine, and glutamate could serve as the substrates to generate fumarate, thus being part
267 of the TCA cycle. Besides, isoleucine turns into the substrates of 2-methyl-acetoacetyl-CoA after
268 several enzymatic steps, which is then converted into acetyl-CoA and propanoyl-CoA *via* acetyl-
269 CoA C-acyltransferase. Acetyl-CoA is an important intermediate, which can entry to the TCA
270 cycle *via* citrate synthase (50). It is also the precursors of fatty acid and polyketides biosynthesis
271 (51). Propanoyl-CoA serves as the critical substrate within propanoate metabolism and can also
272 be used to make lipids (52, 53). On the other hand, methionine can be converted to 2-
273 oxobutanoate, which is also an intermediate of propanoate metabolism. Subsequently, the
274 methylmalonyl-CoA generated in propanoate metabolism enters into the TCA cycle *via* succinyl-
275 CoA. Besides, the key enzymes of glycolysis/gluconeogenesis were found, indicating the
276 potential to produce essential biomass components based on oxaloacetate from TCA cycle.

277

278 Evidence indicates that a source of redox is needed for complete keratin degradation with
279 keratinases (54, 55). Several metabolites such as sulfite were revealed to be associated with
280 efficient keratin degradation (56). Therefore, here the complete metabolic pathway of sulfate
281 reduction was mapped in the genome, which shows that the potential to create a redox
282 environment needed for keratinases is indeed present. Following the development of sequencing
283 technologies, increasing genomes of keratinolytic species have been unveiled, which provide a
284 genomic perspective to reveal the molecular keratinolytic mechanisms. For instance, metabolic
285 pathways related to keratin degradation such as enzymolysis and reduction of disulfide bonds
286 were clarified through uncovering the genetic basis of microbial genomes (57). The complex
287 keratinolytic processes of *Streptomyces* sp. included protease secretion, iron uptake, spore
288 formation, and resuscitation were recently revealed from a genome view (58). Our results are in
289 line with the notion that a redox environment is indeed required for efficient keratinolytic
290 activity to occur. It is expected that the integrated metabolic pathways associated with
291 keratinolytic processes will be deciphered along with more genomes sequencing and biochemical
292 studies of relevant metabolic pathways.

293

294 **Conclusion**

295 In this work, the genomes from four *Chryseobacterium* spp. with keratinolytic activity were
296 analyzed. Common and unique secondary metabolite gene clusters were mined from
297 *Chryseobacterium* spp. genomes, suggesting the potential to generate high value metabolites
298 using keratin-rich wastes as the nutrient sources. Therefore, the use of these microorganisms
299 could be an alternative way to valorize keratinous materials through microbial conversion.

300 Furthermore, the metabolic pathways of keratin degradation from *Chryseobacterium* sp. KMC2
301 was studied from a genomic viewpoint. Nevertheless, there are still unknowns to link both
302 metabolic pathways of keratinous utilization and the natural products biosynthesis.
303 Understanding these connected pathways and their regulation will contribute to developing
304 synthetic biology approaches to boost high value-added products from microbial keratin
305 degradation.
306
307 **Materials and Methods**
308
309 **DNA preparation**
310 *Chryseobacterium* sp. KMC2 was isolated and identified from a keratinolytic microbial
311 consortium enriched from a soil sample (19, 27, 28). The keratinolytic capacity of
312 *Chryseobacterium* sp. KMC2 was evaluated as the keystone strain among the microbial
313 consortium, which was able to degrade keratin materials efficiently (27). *Chryseobacterium* sp.
314 KMC2 was inoculated to LB medium, and cultured overnight (200 rpm, 30 °C). Two milliliters
315 of the suspension were centrifuged and collected to prepare the DNA extraction, performed by
316 using by FAST Soil DNA Kit (MP Biomedicals, United States) according to the manufacturer's
317 instructions.
318
319 **Genome sequencing, assembling, and functional annotation**
320 The genome sequencing was performed by an Illumina Miseq instrument at the University of
321 Copenhagen by using DNA Library Preparation Kits v2 (2 × 250 bp), according to the
322 manufacturer's instructions. Raw reads were treated and assembled to contigs on CLC Genomic

323 Workbench 8.5.1. The obtained contigs were validated using QUAST 4.5 (59). Genes were
324 predicted from the contigs and further annotated with Prokka v1.14.5 (60). Predicted genes from
325 contigs were submitted to eggNOG 5.0 database to obtain an integrated functional annotation
326 and classification (61).
327
328 **Whole-genome phylogenetic analysis**
329 To determine the phylogenetic origin of *Chryseobacterium* sp. KMC2 in the *Chryseobacterium*
330 genus, the whole-genome sequences of 11 publicly available *Chryseobacterium* spp. were
331 downloaded from NCBI database to construct a phylogenetic tree. The whole-genome sequence-
332 based phylogenetic tree was inferred by using an online pipeline: The Reference sequence
333 Alignment based Phylogeny builder (REALPHY 1.12) (62), based on the merge reference
334 alignments. Then the visualization of the phylogenetic tree was generated by iTOL v5 (63).
335
336 **Secondary metabolite gene cluster detection and annotation**
337 Assembled contigs of four *Chryseobacterium* spp. were uploaded to antiSMASH 5.0 secondary
338 metabolite genome mining web platform (21). Predicted secondary metabolites gene clusters
339 from *Chryseobacterium* sp. KMC2 were compared with other keratinolytic *Chryseobacterium*
340 strains. Gene annotation of each cluster from *Chryseobacterium* sp. KMC2 was performed by
341 Prokka v1.14.5 (60) and BLASTP with the NCBI database. The best match sequencing ID was
342 recorded for the annotated genes. Synteny and features of conservative secondary metabolite
343 gene clusters were analyzed by using Easyfig 2.2.2, showing the similarity of gene sequences
344 (64). Feature comparison of amino acid sequences and motifs from core synthetic genes were

345 analyzed by using Clustal Omega (65) to get the multiple sequence alignment and using
346 Seq2logo to generate sequence logo (66).
347
348 **Metabolic networks construction**
349 The genomes of *Chryseobacterium* sp. KMC2 and other three *Chryseobacterium* spp. were
350 submitted to GhostKOALA (67) to obtain the KO number for each gene, then genes were
351 assigned to different metabolic pathways and functional categories. Following the metabolic
352 networks construction of *Chryseobacterium* sp. KMC2 was achieved through mapping the
353 annotated enzyme genes to KEGG (68) reference pathway and Biocyc database (69) manually.
354
355 **Data availability**
356 Reference genomes were downloaded from the NCBI database: *Chryseobacterium camelliae*
357 strain Dolsongi-HT1 (GenBank: GCA_002770595.1), *Chryseobacterium gallinarum* strain DSM
358 27622 (GenBank: GCA_001021975.1), *Chryseobacterium* sp. P1-3 (GenBank:
359 GCA_000738495.1), *Chryseobacterium gleum* NCTC11432 (GenBank: GCA_900636535.1),
360 *Chryseobacterium bernardetii* H4638 (GenBank: GCA_003815955.1), *Chryseobacterium*
361 arthrosphaerae FDAAGOS 519 (GenBank: GCA_003812705.1), *Chryseobacterium indologenes*
362 FDAARGOS 337 (GenBank: GCA_002208925.2), *Chryseobacterium joostei* DSM 16927
363 (GenBank: GCA_003815775.1), *Chryseobacterium glaciei* IHBB 10212 (GenBank:
364 GCA_001648155.1), *Chryseobacterium carnipullorum* F9942 (GenBank: GCA_003815855.1),
365 and *Chryseobacterium* sp. SNU WT5 (GenBank: GCA_007362475.1). Raw sequencing data
366 were deposited in the Sequence Read Archive (SRA) database under the BioProject number
367 PRJNA686768 with an accession number SRR13278108. The assembled genome sequence

368 of *Chryseobacterium* sp. KMC2 has been deposited at DDBJ/ENA/GenBank under the accession
369 JAESIT000000000.
370
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375
376
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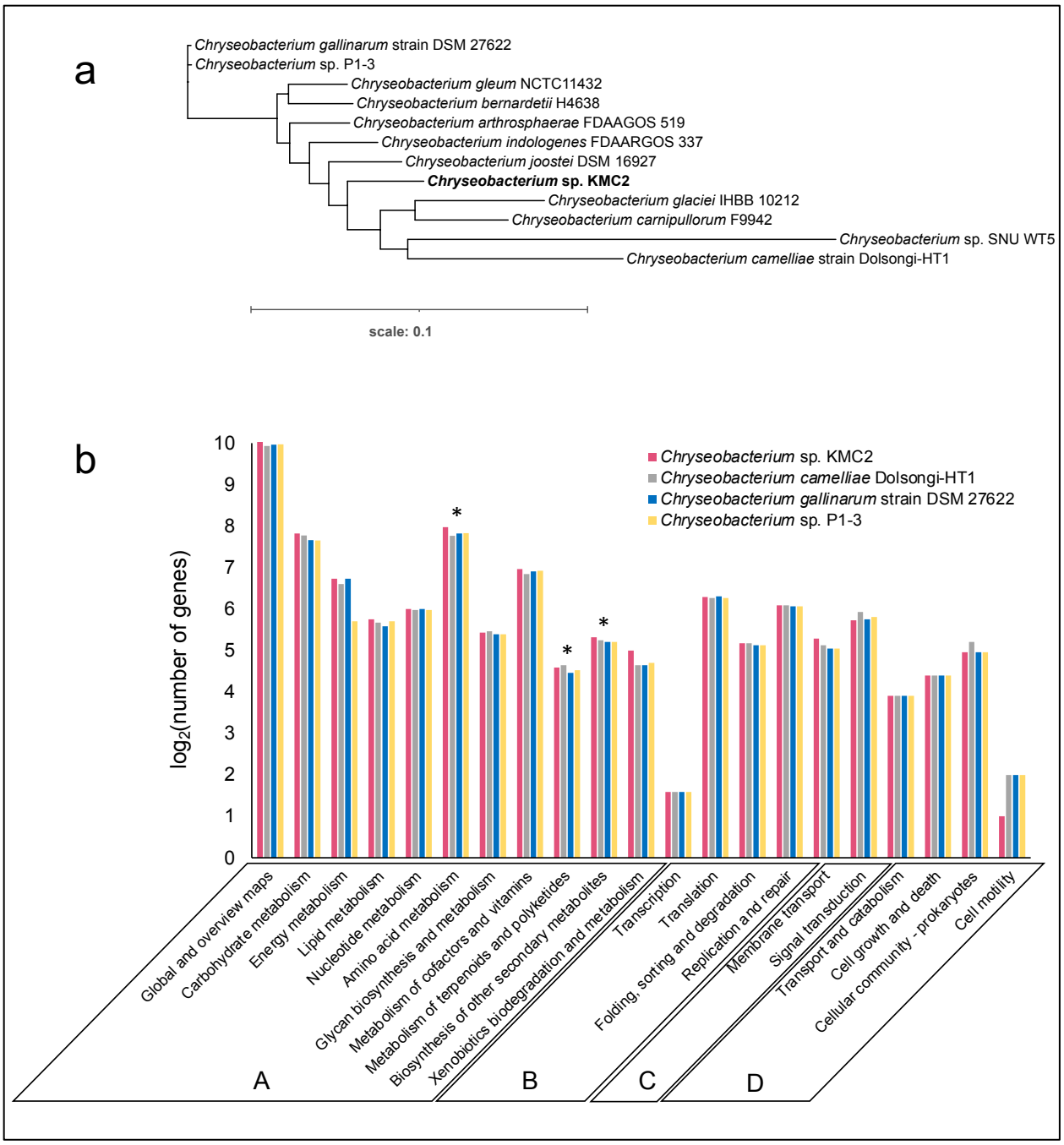

Fig. 1. Analysis of the *Chryseobacterium* spp. genomes. (a). The whole-genome sequence-based phylogenetic tree of *Chryseobacterium* spp., constructed by REALPHY 1.12 (62), based on the merge reference alignments of all genomes. Branch length represents divergence. (b). Comparison of KEGG function classification amongst four *Chryseobacterium* spp. genomes. Functional categories: Metabolism (A), Genetic information processing (B), Environmental

575 information processing (C), and Cellular processes (D). The stars show the sub-categories:
576 Amino acid metabolism, metabolism of terpenoids and polyketides, and biosynthesis of other
577 secondary metabolites.
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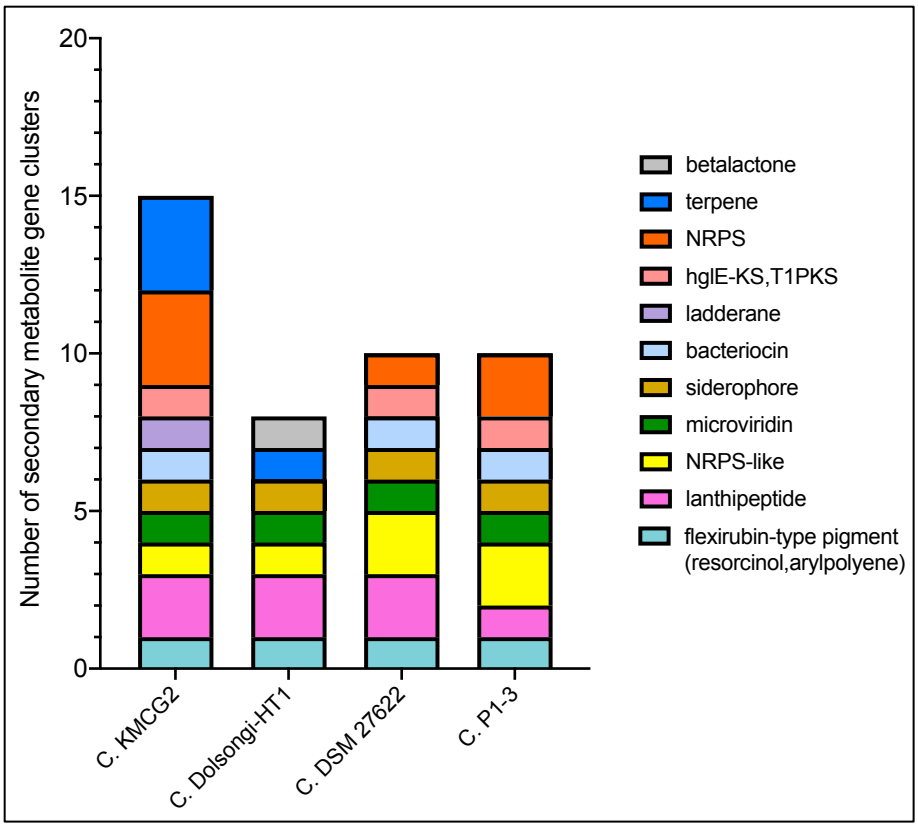

593 **Fig. 2. Composition of secondary metabolite gene clusters from four *Chryseobacterium* spp.**

594 **genomes.**

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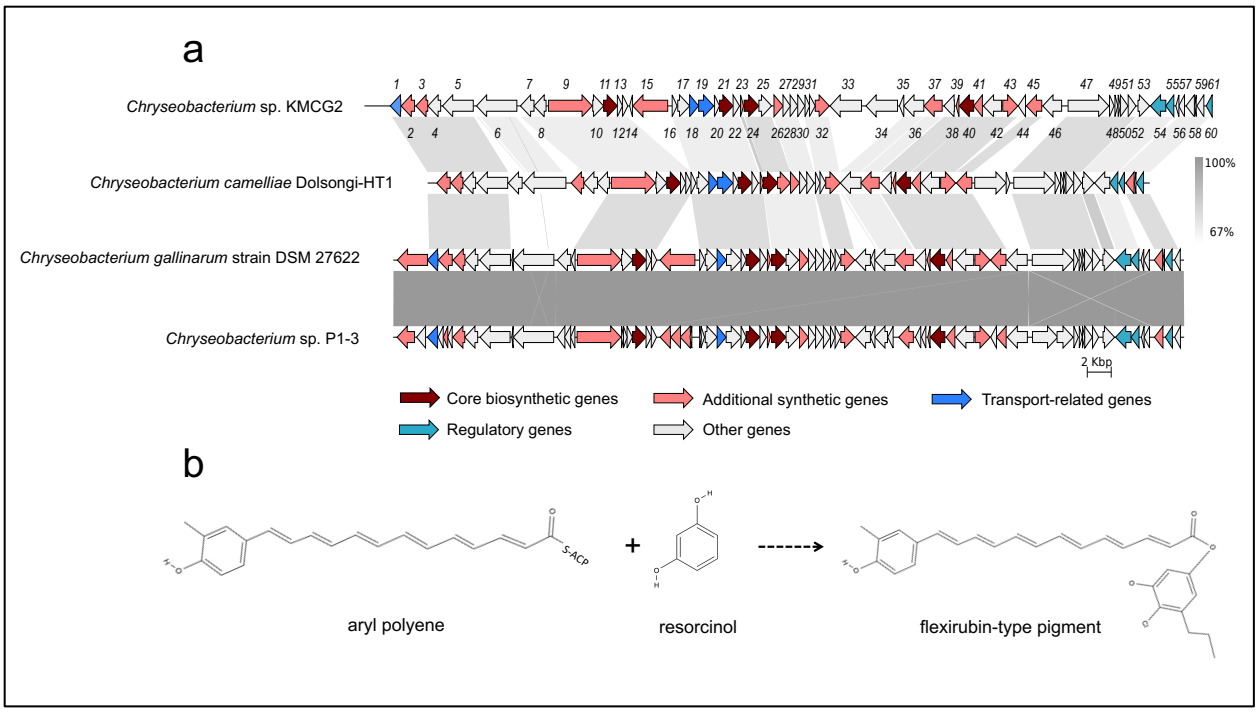

Fig. 3. Flexirubin-type pigment gene cluster from four *Chryseobacterium* spp. genomes. (a). Synteny analysis and features of flexirubin-type pigment gene cluster in *Chryseobacterium* spp. genomes. (b). The proposed biosynthetic reaction of flexirubin-type pigment. The detailed description of each gene can be found in Table S1.

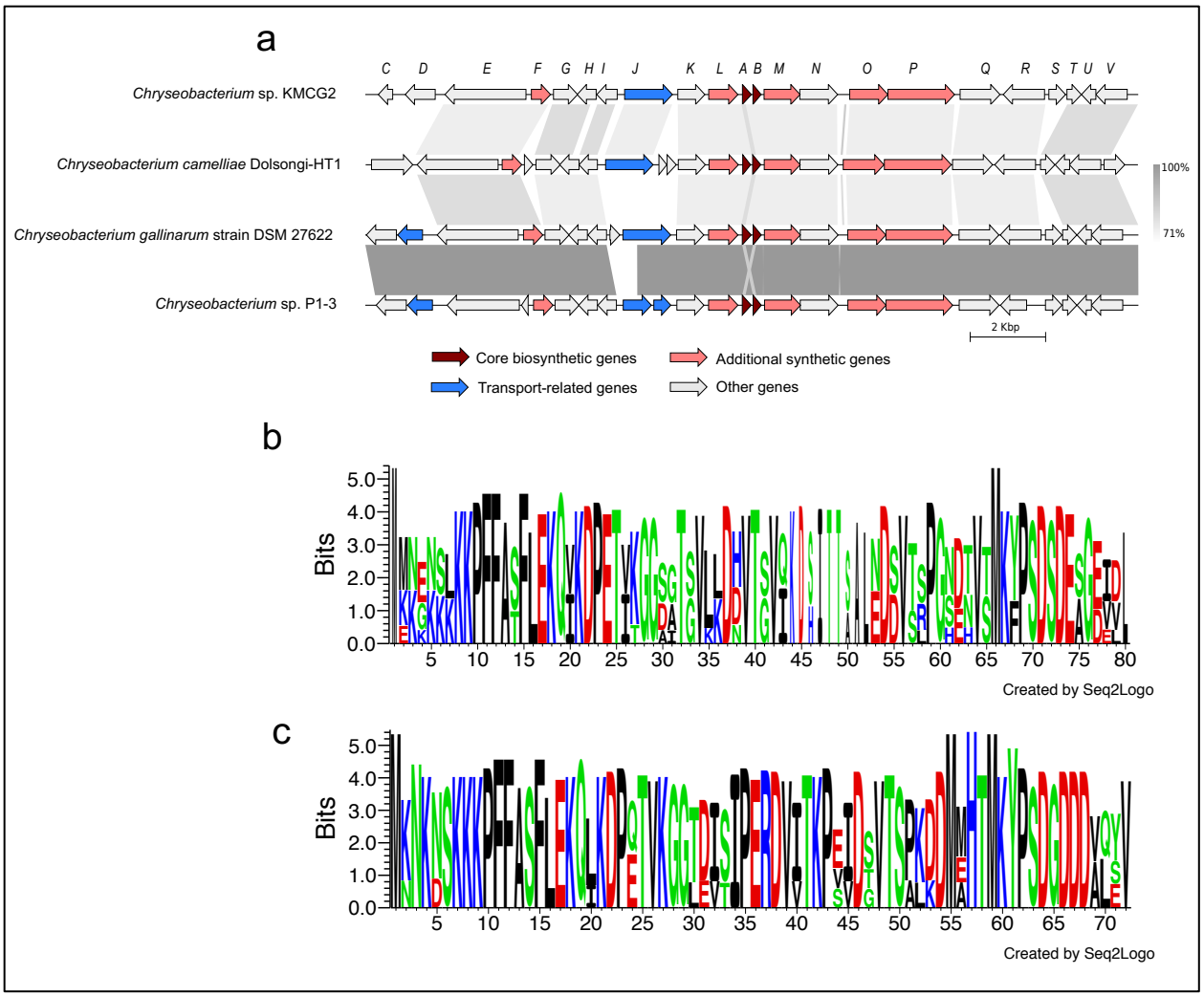

Fig. 4. Microviridin gene cluster from four *Chryseobacterium* spp. genomes. (a). Synteny analysis and features of microviridin gene cluster in *Chryseobacterium* spp. genomes. (b). Amino acid sequence comparison of mdnA from four *Chryseobacterium* spp. genomes. (c). Amino acid sequence comparison of mdnB from four *Chryseobacterium* spp. genomes. The detailed description of each gene can be found in Table S2.

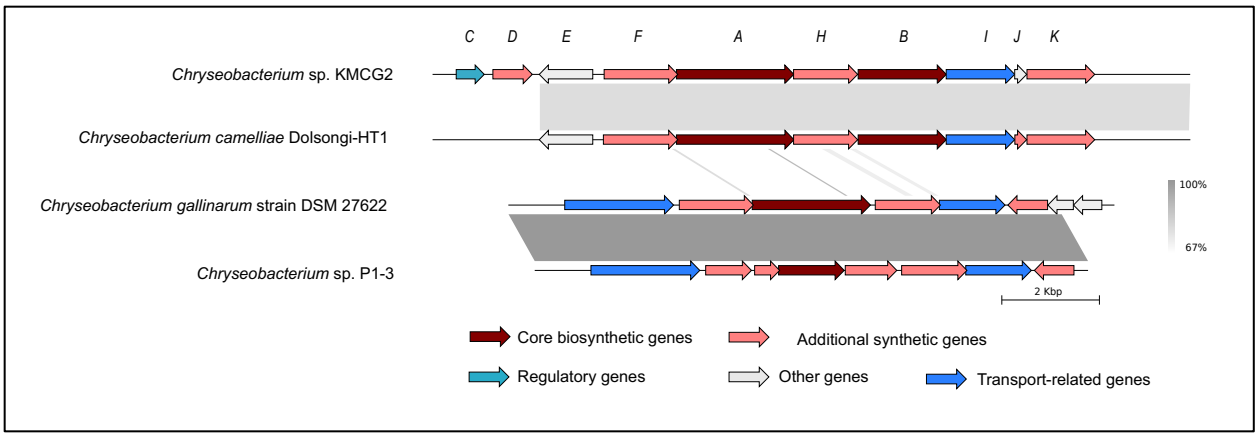

Fig. 5. Siderophore gene cluster from four *Chryseobacterium* spp. genomes. The detailed description of each gene can be found in Table S3.

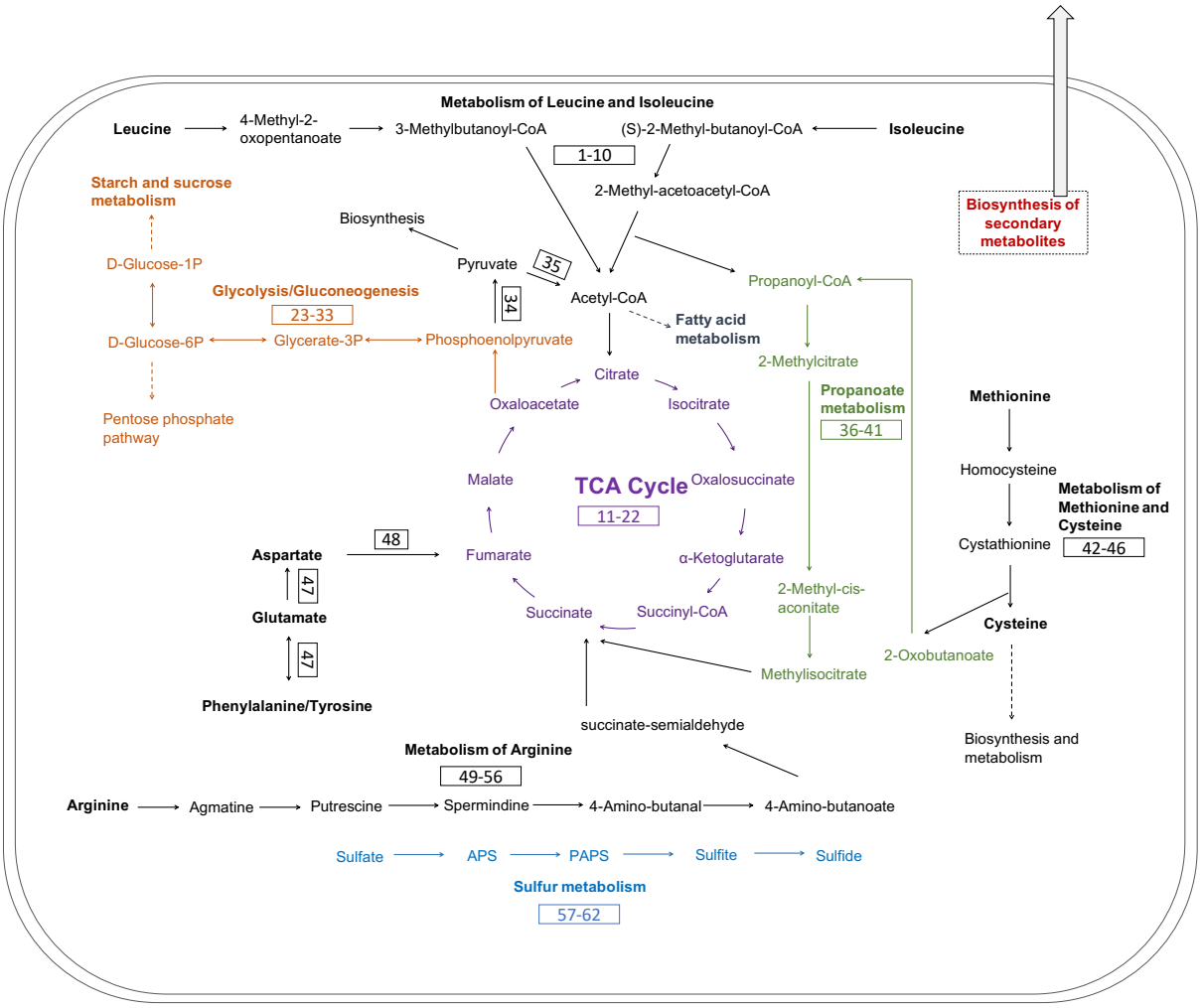

Fig. 6. Metabolic pathways reconstruction of keratin utilization in *Chryseobacterium* sp. KMC2 genome. It includes amino acid metabolism, TCA cycle, glycolysis/gluconeogenesis propanoate metabolism, and sulfur metabolism. The number in the box represents the gene related to the metabolic pathway. The detailed description of each gene can be found in Table S7.

Table 1. Features comparison of four *Chryseobacterium* spp. genomes.

Parameters	*Chryseobacterium* sp. KMC2	*Chryseobacterium camelliae* Dolsongi-HT1	*Chryseobacterium gallinarum* strain DSM 27622	*Chryseobacterium* sp. P1-3
Total length (bp)	5.276.159	4.376.354	4.633.632	4.628.764
Contigs	63	1	1	45
N50 (bp)	231.784	4.376.354	4.633.632	342.512
GC content (%)	36.33	41.80	37.30	37.02
Gene	4.773	3.999	4.182	4.087
CDS	4.692	3.881	4.033	3.119