Engineering *Elizabethkingia meningoseptica sp.* F2 for Vitamin K2 production guided by genome analysis.

Qiang Yang
Chinese Acadamy of science
zhiming zheng (zhengzhiming2014@163.com)
Hefei Institutes of Physical Science, Chinese Academy of Sciences https://orcid.org/0000-0002-0550-8087
Hui Liu
Chinese Acadamy science
Peng Wang
Chinese Acadamy of science
Li Wang
Chinese Acadamy science
Xiaowen Sun
Chinse Acadamy of science
Wenfeng Ni
Chinese Acadamy of science
Han Wang
Chinese Acadamy of Science
Hengfang Tang
Chinese Acadamy of science
Genhai Zhao
Chinese Acadamy of science

Research

Keywords: Vitamin K2, Elizabethkingia meningoseptica, Comparative genomics, metabolic network analysis

Posted Date: May 1st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-25351/v1

Abstract

Background

The species in family *Elizabethkingia meningoseptica* are interesting strain for investigating Vitamin K2 metabolic analysis. However, their genomic sequence, metabolic pathway, potential abilities, and evolutionary status are still unknown.

Results

This study therefore aimed to perform a genome sequencing of *Elizabethkingia meningoseptica sp. F2* and further accomplished comparative analysis with other Vitamin K2 strains reveals overall identifying its unique/shared metabolic genes across genomes. The 3,874,794–base pair sequence of *Elizabethkingia meningoseptica sp. F2* is presented. Of 3,539 genes annotation was applied. Results of synteny block demonstrated *Elizabethkingia meningoseptica sp. F2* shares high levels of synteny with *Elizabethkingia meningoseptica ATCC 13253* and *Elizabethkingia meningoseptica NBRC 12535*. Identification of Vitamin K2 metabolic pathway in *Elizabethkingia meningoseptica sp. F2* were also accomplished. In addition, *Elizabethkingia meningoseptica sp. F2* was resistant to gentamicin, streptomycin, ampicillin and caramycin, consistent with the presence of multiple genes encoding diverse multidrug efflux pump protein in the genome. Furthermore, By co-overexpression experiments of MenA and MenG, we showed that Vitamin K2 content was enhanced by 37% compared with control strain.

Conclusions

The genome analysis of *Elizabethkingia meningoseptica sp. F2* in conjunction with the comparative metabolic pathways analysis among the *E.coli, Bacillus subtilis* and *Streptomyces* provided a useful information on the Vitamin K2 biosynthetic pathway and other related pathways at systems level.

Background

Vitamin K is a group name for a family of related compounds, generally subdivided into phylloquinone K1(vitamin K1) and menaquinone K2 (Vitamin K2)[1]. Vitamin K2 is an essential lipid-soluble vitamin that play important roles in a number of vital physiolog-ical processes including haemostasis, calcium and bone metabolism, as well as cell growth regulation[2, 3]. In addition, it can also functions as an electron carrier in prokaryotes[4]. Therefore, Vitamin K2 is receiving increasing attention in the domain of nutritional supplements for humans. Vitamin K2 refers to a group of menaquinones (MKs) varying in side chain length. Different forms of MKs are written as MK-n(Fig. S1), where n stands for the number of isoprene units in the side chain[5–7]. Till date, some of the most widely examined Vitamin K2 production microorganisms include *Bacillus subtilis natto*, *Bacillus subtilis168*, *Elizabethkingia meningoseptica*, *Escherichia coli*, and others.

Isolates of the genus *Chryseobacterium*, formerly *Flavobacterium*, named for its yellow pigment, are non-motile, non-fastidious, Gram-negative bacilli[8].The genus *Elizabethkingia* was established in 2005 based on an analysis of 16S rRNA gene sequences from strains within the family *Flavobacteriaceae*[9]. *E. meningoseptica* are most frequently isolated from soil, saltwater and freshwater and from dry and municipal water supplies including those which have been adequately chlorinated[10]. It has also been reported that *E. meningoseptica* is resistant to multiple antibiotics, such as extended-spectrum β-lactam agents and aminoglycosides [11, 12] .

Mutational improvement of cells has contributed to the development of microbial processes for the MK production of *Elizabethkingia meningoseptica*[13–16]. Modular pathway engineering is another effective method for improving the biosynthesis of specific chemicals[17, 18]. Nevertheless, the genome and Vitamin K2 relevant metabolic pathway of *Elizabethkingia meningoseptica sp. F2* remain unclear. Despite the recent publication of several complete genomes of *Elizabethkingia meningoseptica* at NCBI database, no studies have yet used whole genome information more extensively in order to understand the Vitamin K2 biosynthetic pathway of *Elizabethkingia meningoseptica*. Genome sequencing and comparative analysis can help identify shared and species-specific Vitamin K2 in closely-related species.

In this study, We firstly studied *Elizabethkingia meningoseptica* F_2 using whole-genome sequencing (WGS) and compared it with other available strain genomes(microorganisms can be used to biosynthesize Vitamin K2). Specifically, the orthologous genes involved in the Vitamin K2 metabolic pathway were identified. Subsequently, the identification of Vitamin K2 metabolic pathway in *Elizabethkingia meningoseptica sp. F2* were also accomplished. Moreover, the recombinant expression of the synthetic menaquinone pathway operons of *Elizabethkingia meningoseptica* F_2 increased the production of Vitamin K2 in our engineered strains. The information and materials presented in this study will be of great use in improving and modifying *Elizabethkingia meningoseptica* and other strains for the production of Vitamin K2.

Materials And Methods

Bacterial strains, plasmids, media and cultivation.

All microorganisms and plasmids used in this study are listed in Table S1. *E. coli* strain *DH5α* was used for plasmids construction and propagation. *Elizabethkingia meningoseptica sp. F2* (CCTCC AB2011070) were as industrial strain for Vitamin K2 production. Plasmids pMBIS and pTric-hisA were used for pathway construction. Luria-Bertani (LB) media containing 10 g/L NaCl, 10 g/L tryptone and 5 g/L yeast extract was used for plasmid propagation. The media and cultivation of *Elizabethkingia meningoseptica sp. F2* were performed as previously described[19].

For extraction from bacterial biomass, bacteria were harvested by centrifugation at 12000 rpm for 15 min. The supernatant was discarded. For Vitamin K2 extraction from was extracted from the fermentation media, the method was adapted from Wei et al[20]. Vitamin K2 analysis was performed by

HPLC(Shimadzu, Kyoto, Japan) using eclipse plus a C18 column (Shimadzu, 250 mm x 4.6 mm ID), and aliquots of 20 μL were injected manually using a loop injection valve (Shimadzu). In addition, the temperature of the column was maintained at 35 °C. Mobile phase consisted of methanol and dichloromethane (4:1, v/v) with the flow rate of 1 mL min^{-1}. The UV–Vis detector was operated at 248 nm for menaquinones.

16 s rRNA sequencing and phylogenetic analysis.

The strain *Elizabethkingia meningoseptica sp. F2* was further submitted to the Sangon Biotech (Shanghai) Co., Ltd, China for 16 s rRNA sequencing. Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product was carried out. We retrieved from NCBI the 16S rRNA gene sequences of seven strains of *Elizabethkingia*. Phylogenetic tree was established based on all 16S rRNA gene sequences of *Elizabethkingia meningoseptica sp. F2* and reference strains to infer the phylogenetic relationships of *Elizabethkingia* strains. The phylogenetic tree was made with NCBI BLAST pairwise alignments by using the method of Fast Minimum Evolution method.

Genome sequencing and assembly

The *Elizabethkingia meningoseptica sp. F2* genome was sequenced using a PacBio RS II platform and Illumina HiSeq 4000 platform at the Beijing Genomics Institute (BGI, Shenzhen, China). Four SMRT cells Zero-Mode Waveguide arrays of sequencing, were used by the PacBio platform to generate the subreads set. PacBio subreads (length < 1 kb) were removed. The program Pbdagcon (https://github.com/ Pacific Biosciences /pbdagcon) was used for selfcorrection. Draft genomic unitigs, which are uncontested groups of fragments, were assembled using the Celera Assembler against a highquality corrected circular consensus sequence subreads set. To improve the accuracy of the genome sequences, GATK (https://www. broadinstitute.org/gatk/) and SOAP tool packages(SOAP2, SOAPsnp, SOAPindel) were used to make single-base corrections. To trace the presence of any plasmid, the filtered Illumina reads were mapped using SOAP to the bacterial lasmid database (http://www.ebi.ac.uk/genomes/plasmid.html, last accessed July 8, 2016).

Genome annotation and component prediction

Gene prediction was performed on the *Elizabethkingia meningoseptica sp. F2* genome assembly by glimmer3(http://www.cbcb.umd.edu/software/glimmer/) with Hidden Markov models. tRNA, rRNA and sRNAs recognition made use of tRNAscan-SE [21], RNAmmer, and the Rfam database. The tandem repeats annotation was obtained using the Tandem Repeat Finder (http://tandem.bu.edu/trf/trf.html), and the minisatellite DNA and microsatellite DNA selected based on the number and length of repeat units. Prophage regions were predicted using the PHAge Search Tool (PHAST) web server (http://phast.wishartlab.com/) and CRISPR identification using CRISPR Finder.

The best hit abstracted using Blast alignment tool for function annotation. Several databases were used for genome annotation and gene function prediction, including Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Clusters of Orthologous Groups (COG), Non-Redundant Protein Database databases (NR), Transporter Classification Database (TCDB), Swiss-Prot (http://www.gpmaw.com/html/swiss-prot.html), EggNOG, and TrEMBL (https://www.uniprot.org/). Four databases for pathogenicity and drug resistance analysis. Virulence factors and resistance gene were identified based on the core dataset in VFDB (Virulence Factors of Pathogenic Bacteria) and ARDB (Antibiotic Resistance Genes Database) database, other two are PHI (Pathogen Host Interactions) and (Carbohydrate-Active enZYmes Database).

Comparative genomics

The synteny of *Elizabethkingia meningoseptica.ATCC13253*(GenBank ACCESSION number.ASAN00000000), *Elizabethkingia meningoseptica.NBRC12535*(GenBank ACCESSION number BARD00000000), *Bacillus subtilis natto*(GenBank ACCESSION number GCA_000209795.2), *E.coli.K-12*(GenBank ACCESSION number: NC_000913) and *Elizabethkingia meningoseptica sp. F2*(all of those strain can synthesize Vitamin K2) was performed using MUMmer and BLAST Core/Pan genes of *Elizabethkingia meningoseptica sp. F* and other four strains were clustered by the CD-HIT rapid clustering of similar proteins software with a threshold of 50% pairwise identity and 0.7 length difference cutoff in amino acid.

Resistance to tolerance test of Elizabethkingia meningoseptica sp. F2.

Previous studies revealed that the resistome of *E. meningoseptica* contain resistance genes and is resistant to most antimicrobial agents commonly[22–24]. *Elizabethkingia meningoseptica sp. F2* were tested for antibiotic susceptibility refering to the Kirby-Bauer disk diffusion method as previously described[25]. To determine antimicrobial resistance concentrations, the different concentrations of six antimicrobial agents were assessed. The ranges of antimicrobial agents were as follow: Ampicillin (50 mg/L ~ 200mgL), Caramycin (50 mg/L ~ 200mgL), Tetracycline(50 mg/L ~ 150mgL), and Chloramphenicol (50 mg/L ~ 150mgL), Gentamicin(50 mg/L ~ 200mgL), Streptomycin(50 mg/L ~ 200mgL). The cultures were re-suspended in sterile 0.85% saline and adjusted to OD600 nm of 0.5 in a Biowave CO8000 Cell Density Meter. Plates containing 20 ml of solidified medium and several different concentrations of the antimicrobial were prepared on the day of the experiment. The plates were incubated at 37℃overnight. The positive growth control well contained no antimicrobial agent.

DNA manipulation.

General DNA manipulation was conducted according to standard protocols. DNA purification and plasmid isolation kits were purchased from Sangon Biotech (Shanghai, China). DNA manipulation enzymes and restriction enzymes were provided by Thermo Inc(ThermoScientific, USA). Primer sequences are listed in Table.S2. The structures of the plasmids are shown in Fig. S2. Identification of metabolic bottlenecks in the quinonoid ring modification pathway were done. The MenA ~ MenG gene

was amplified from *Elizabethkingia meningoseptica sp. F2*. genome using primers as described in Table S2. And then PCR product was digested by *Xho*I and *Sac*I and then inserted into the corresponded sites of pMBIS, formed pMBISMenA ~ pMBISMenG. The combinatorial overexpression of homologous genes were expressed under control of the strong induced promoter Ptrc in plasmid pTrc-hisA. All the plasmids were transferred into *Elizabethkingia meningoseptica sp. F2* by heat shock transformation (Table S1). Then the constructs were confirmed by Sanger sequencing and all the strains were validated by colony PCR.

Nucleotide sequence data deposition.

16S rRNA sequence and genome sequences of *Elizabethkingia meningoseptica sp. F2* were deposited in the NCBI GenBank under the following accession numbers: MT150737 (https://www.ncbi.nlm.nih.gov/nuccore/MT150737) and CP050128

(http://www.ncbi.nlm.nih.gov/nuccore/CP050128), respectively.

Result

Phylogenetic and basic characteristic analysis of Elizabethkingia meningoseptica sp. F2.

A 16S rRNA sequence alignment of seven *Elizabethkingia* from NCBI database and the nearly full-length sequence(1445 bp) of the 16S rRNA gene of *Elizabethkingia meningoseptica sp. F2* was carried out. The results showed that *Elizabethkingia meningoseptica sp. F2* cluster with the *Elizabethkingia meningoseptica ATCC 13253*(NR_042267.1, NR_115201.1), *Elizabethkingia meningoseptica LMG 12279*(NR_115136.1) and *Elizabethkingia meningoseptica NBRC 12535*(NR _113592.1) in one clade (Fig. 1). Our phylogenomic tree also showed a distinct separation of strains of into there different clusters. Moreover, this tree obviously indicated that *Elizabethkingia meningoseptica* strains are genetically distinct from the outgroup *Elizabethkingia anophelis* and *Elizabethkingia miricola*. The morphological appearance of cells was also studied using scanning electron microscopy as our previously described[19]. Similar results were observed that the cells are Gram-negative, non-motile, (0.5 × 1.0–2.0 μm, Fig.S3)non-spore-forming rods[26].

Sequencing and gene annotation of the Elizabethkingia meningoseptica sp. F2 genome.

The genome sequence of *Elizabethkingia meningoseptica sp. F2* was obtained by whole genome sequencing. The principal features of the *Elizabethkingia meningoseptica sp. F2* chromosome are summarized in Table 1. The genome in *Elizabethkingia meningoseptica sp. F2* had a length of 3,874,794 bp, a GC-content 70.7%. Genome annotation yielded 3,539 genes and 62 RNA genes, respectively. At least 276 Repeat, 1 CRISPR and 1 prophage were predicted in *Elizabethkingia meningoseptica sp. F2*. Protein-coding genes account for 87.42% of the genome, 0.5647% encodes stable RNAs, and 0.8087% consists of noncoding repeats, 11.2% for regulatory and other functions. The function annotation is accomplished by analysis of protein sequences by aligning genes with different databases to obtain their corresponding annotations. we have finished COG, KEGG databases annotation,

the result of COG Database Annotation was listed in Figure S4. According to the analysis of the sequencing samples, Circos[27] software was used to display the genome(Fig. 2), ncRNA, repetitive sequences, annotation information, methylation, GC content, GC skew and other information on the genome of sequencing strains.

Table 1
Overview of chromosomal features of the Elizabethkingia meningoseptica sp. F2 genome

Linear chromosome Property	
Length (bp)	3,874,794
GC content (%)	37.57
Total Number of genes	3,539
Total length of gene	3387246
The proportion of gene length in genome	87.42(%)
RNA	49
tRNA	12
rRNA(16S-23S-5S)	7
sRNA	168
Repeat	61
Tandem repeat	47
Minisatellite DNA	1
Microsatellite DNA	1
Prophage	88
CRISPR	13
VFDB	
ARDB	
Note: Virulence factors of pathogenic bacteria (VFDB) database, Antibiotic resistance(ARDB) genes database	

Comparative analysis of Elizabethkingia meningoseptica sp. F2 with Eme. ATCC12535 and other strains.

We compared the genomes of *Eme.ATCC13253*, *Eme.NBRC12535*, *B.subtilis.natto, E.coli.K-12* to that of *Elizabethkingia meningoseptica sp. F2* (All of microorganisms can be used to biosynthesize Vitamin K2) to identify any differences in their genomic content. Structure variation analysis could detect the evolution of homology genomes, for example, the location variations of gene clusters with similar function. Nucleic acid level synteny of those five strains (Fig. 3, S5). The level of vertical colour filling in

Eme.ATCC13253 and *Eme.NBRC12535* was the highest, which revealed the close relative of them and the absence of any species-specific regions. This result also supports the close relationship between these three strains. While whole-genome alignments of *B.subtilis.natto* and *E.coli.K-12* to that of *Elizabethkingia meningoseptica sp. F2* revealed without any levels of synteny. Similar results are observed from the distribution of protein identity of *Eme.ATCC13253*, *Eme.NBRC1253*, *B.subtilis.natto*, *E.coli.K-12* with *Eme.*F_2(Fig. S6). The clustering calculated with the COG function classification data in dispensable gene heat map(Fig. 4) of those five strain, showed that significant inter-group difference among *Elizabethkingia meningoseptica sp. F2* and *Elizabethkingia meningoseptica.ATCC12535*, *Eme.NBRC12535* strains.

A genome wide comparison of COGs in different strains provides insight into gene function, gene structure, and molecular evolution of genomes. The COG analysis of *Elizabethkingia meningoseptica sp. F2* strains was compared with four other strains (*Eme.ATCC13253, Eme.NBRC1253, B.subtilis.natto*,, and *E.coli.K-12*) that have different numbers of categories genes. Venn diagram showing the distribution and number of core, dispensable and strains pecific genes of the five strains(Fig. 5). Each ellipse is labelled with the name of its representative strain. The pan-genome for the five analyzed strains contained 53 core genes. The number of strain-specific genes are 201, 13, 5, 3593, and 3599 for *Eme*, *Eme.ATCC13253, Eme.NBRC1253, B.subtilis.natto*, and *E.coli.K-12*, respectively.

Identification of Vitamin K2 metabolic pathway in Elizabethkingia meningoseptica sp. F2.

According to the results of sequencing annotation(KEGG database), all genes, encoding key enzymes that are required for Vitamin K2 (including shikimate pathway, menaquinone pathway and mevalonate pathway), have been identified in *Elizabethkingia meningoseptica sp. F2* genomes. As can be seen in Fig. 6, S7, the biosynthetic pathway of Vitamin K2 was categorized into there modules, namely, Module I: shikimate (SA) pathway, Module II: menaquinone pathway, there are seven pathway enzymes, designated as MenF (menaquinone-specific isochorismate synthase), MenD(2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase/2-oxoglutarate decarboxylase), MenH(2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase), MenC(osuccinylbenzoate-CoA synthase), MenE(o-succinylbenzoic acid-CoA ligase), MenB(naphthoate synthase), MenI(1,4-dihydroxy-2-naphthoyl-CoA hydrolase), MenA(1,4-dihydroxy-2-naphthoate octaprenyltransferase), MenG and UbiE(Q-8/MK-8 biosynthesis methyltransferase), encoding the head structure biosynthesis enzymes that are involved in the Vitamin K2 biosynthetic pathway starting from isochorismate. Module III: mevalonate pathway(Gene symbols and the enzymes they encode: *atoB*, acetoacetyl-CoA acetyltransferase; *HMGS*, HMG-CoA synthase; *HMGR*, HMG-CoA reductase; *MK*, mevalonate kinase; *MVD1*, mevalonate pyrophosphate decarboxylase; *idi*, IPP isomerase.), and Module IV: ubiquinone modification pathway: only two genes encoding UbiA (4-hydroxybenzoate octaprenyltransferase) and UbiE (Q-8/MK-8 biosynthesis methyltransferase) were found. Our data also support the fact of absent coenzyme Q in *Elizabethkingia meningoseptica sp. F2*(Fig.S7, S8).

The analysis of Elizabethkingia meningoseptica sp. F2 antibiotic tolerance.

Antibiotic susceptibility testing performed on the *Elizabethkingia meningoseptica sp. F2* strain revealed resistance to ampicillin (0 ~ 200mgL), caramycin (0 ~ 200mgL), tetracycline(0 ~ 150mgL), chloramphenicol (0 ~ 150mgL), gentamicin(0 ~ 200mgL), streptomycin(0 ~ 200mgL), and sensitivity to chloramphenicol (100 mg/L ~ 150mgL) and tetracycline(75 mg/L ~ 150mgL). Therefore, The chloramphenicol(> 100 mg/L)and tetracycline(> 75 mg/L) can be used as selection markers in subsequent experiments(Table 2). Our results also show that has some resistance genes are found in *Elizabethkingia meningoseptica sp. F2* genomes(Figure S9), including those encode TolC family protein, multidrug ABC transporter, MFS transporter, multidrug SMR transporter, RND family efflux transporter MFP subunit, bleomycin resistance protein, outer membrane efflux protein, multidrug transporter, hydrophobic/ amphiphilic exporters etc. This observation is consistent with resistance to antibiotics in our tests.

Table 2
Antibiotic resistance test results of *Elizabethkingia meningoseptica sp.*F_2

Antibiotic name	Category	Concentration			
Ampicillin	β-Lactams	**50**	**100**	**150**	**200**
Caramycin	Aminoglycosides	**50**	**100**	**150**	**200**
Tetracycline	Tetracycline	**50**	**75**	**100**	**150**
Chloramphenicol	Chloramphenicol	**50**	**75**	**100**	**150**
Gentamicin	Aminoglycosides	**50**	**100**	**150**	**200**
Streptomycin	Aminoglycosides	**50**	**100**	**150**	**200**

Table 3
Fermentation results of Elizabethkingia meningoseptica sp. F2 transformants with expression of the Men enzyme

Strains	VK_2 titers (mg/L)	Biomass (DCW, g/L)	VK_2 productivity (mg/g DCW)
FlaMCS	20.28 ± 1.47	9.17 ± 1.15	2.21 ± 0.18
FlaMenA	24.16 ± 1.04	9.45 ± 1.06	2.56 ± 0.17
FlaMenB	21.88 ± 0.96	9.38 ± 0.91	2.33 ± 0.12
FlaMenC	19.76 ± 0.54	8.71 ± 0.75	2.26 ± 0.14
FlaMenD	23.05 ± 1.19	9.93 ± 1.64	2.32 ± 0.34
FlaMenE	24.12 ± 1.76	10.03 ± 1.30	2.40 ± 0.22
FlaMenF	23.81 ± 0.51	9.87 ± 0.36	2.41 ± 0.08
FlaUbiE/MenG	24.88 ± 0.37	9.95 ± 0.62	2.49 ± 0.13
FlaMenA &MenG	27.80 ± 0.91	9.82 ± 0.87	2.83 ± 0.41
All the data are shown in the table from three independent experiments.			

Increasing expression of the menaquinone pathway.

As discussed in the genome-scale the Vitamin K2 metabolic network analysis section, overexpression of Men genes to increase precursor supply may be a useful strategy to improve Vitamin K2 production. To increase the intracellular concentration of precursor supplied to synthetise the Vitamin K2, a series of constitutive plasmids carrying different Men enzymes, pMBIS_MenA, pMBIS_MenB, pMBIS_MenC, pMBIS_MenD, pMBIS_MenE, pMBIS_MenF and pMBIS_MenG, were constructed for preliminary screening of key enzymes. To test the functionality of the homologous over expression pathway, Vitamin K2 production by recombinant strains was evaluated. The results(Table 3)indicate that constitutive overexpression of Men genes could strengthen metabolic flux flow into target product and slightly promote the accumulation of biomass compared with the control strain. *Elizabethkingia meningoseptica sp. F2* harboring pMBIS_MenC exhibited a slightly decrease in Vitamin K2 production due to the depression of cell mass. These modifications in pMBIS_MenA, pMBIS_MenG resulted in 19.13%, 22% respectively increase in Vitamin K2 production compared with control strain.

The improvements resulting from overexpression of the Men operon motivated us to examine the use of strong and inducible promoters and construct the MenA-MenG operon. And then the menA gene and menG gene was co-expressed in the plasmid pTrc-hisA to synthsis the final product Vitamin K2 from 1,4-Dihydroxy-2-naphthoate (DHNA), the Vitamin K2 production had further improved to 27.8 ± 0.91 mg/L (increased by 37% in comparison to control strain). However, the overexpression of these genes had an unobvious effect on the cell growth. Based on these results, enhancing metabolic flux from DHNA to

menaquinone could be more effective for improving Vitamin K2 yield. Nevertheless, Engineering *Elizabethkingia meningoseptica sp. F2* for Vitamin K2 production still have a lot of work to do. Therefore, the combination between overexpression key genes of menaquinone pathway(Module II) and modification of the mevalonate pathway(Module III) will also be explored in subsequent experiments.

Discussion

The 16S rRNA gene has been used for the classification of microorganism species [28, 29]. In this context, Our strain can certainly be placed among *Elizabethkingia meningoseptica* species. In this study, a high-quality assembled genome of an industrially relevant *Elizabethkingia meningoseptica sp. F2* used for Vitamin K2 production. This was achieved by a hybrid sequencing approach which used a PacBio RS II platform and Illumina HiSeq 4000 platform. Comparative analysis of *Elizabethkingia meningoseptica sp. F2*, *Elizabethkingia meningoseptica sp.NBRC12535* and *Elizabethkingia meningoseptica sp. ATCC13253* revealed highly conserved internal region where most essential genes are located, with similar order and direction in the three species. *Elizabethkingia meningoseptica* species are irreversibly different from *E. coli* and *B.subtilis.natto* species in terms of their genetic properties. The GC content in strain *Elizabethkingia meningoseptica sp. F2*(37.5%) was higher than in *Elizabethkingia meningoseptica sp*. ATCC13253(36.6%), and *Elizabethkingia meningoseptica sp.NBRC12535* (36.4%)(Table S3), indicating their diverse genome evolution under differential selection[30]. Additionally, our results also show that *Elizabethkingia meningoseptica sp. F2* has some virulence factors and resistance genes(Table 1).

We were mostly interested in Vitamin K2 pathway related genes. Hence, Mining of the orthologous genes(KEGG database) involved in Vitamin K2 biosynthesis of other six species was also performed (Fig. 7), a comparative Vitamin K2 metabolic pathway approach was used in this study, in order to understand the gene cluster composition of different strains genome. The results strongly suggest that the mevalonate pathway of *Elizabethkingia meningoseptica sp. F2* is unique. On account of previously metabolic pathway analysis demonstrated that the mevalonate pathway is mainly found in eukaryotes, whereas the MEP pathway is normally found in prokaryotes[31]. Interestingly, The gene menI was identified in the genome of *Elizabethkingia meningoseptica* and *E. coli*, whereas absent in other five strains. Unfortunately, PMK gene was not identified in the genome(Figure.7a). On the other hand, we also found that same emzyme GGPPS(geranylgeranyl diphosphate synthase) catalyze the synthesis of Geranylgeranyl diphosphate from IPP or DMAPP by three reactions. In the past, the MK-4, MK-5 and MK-6 of *Elizabethkingia meningoseptica* were identified as our described[20, 32]. However, only geranylgeranyl diphosphate synthase and octaprenyl diphosphate synthase were identified, the hexaprenyl diphosphate synthase(catalyze farnesyl diphosphate to hexaprenyl diphosphate**)** was not found. As shown in Figure S10a, the mechanism of how to synthetize pentenyl diphosphate is remain unclear in this pathway.

Similar results were observed from the overexpression of 1,4-dihydroxy-2-naphthoate octaprenyltransferase(MenA) in *B. subtilis* 168 and *E. coli* increased the Vitamin K2 content, in which imply that the reaction of converting 1,4-Dihydroxy-2- naphthoate to 2-Demethylmenaquinone plays the

most important role in increasing Vitamin K2 production[33–35]. Therefore, the enzyme MenA was listed as rate-limiting enzymes of the Menaquinone pathway.

It has been reported that *Escherichia coli* could synthesizes a naphthoquinone- type menaquinone-8(MK-8) under anaerobic conditions [35, 36]. Corresponding to this result is IspB(octaprenyl- diphosphate synthase, convert Famesyl-PP to Octaprenyl-PP) was identified (Figure.7b). The 2C-methyl-d-erythritol-4-phosphate (MEP) pathway consists of eight enzyme-catalyzed reactions in *Escherichia coli*[37]. Further details about MEP biosynthetic pathway can be found in FigureS10b. MEP pathway are not sufficient for the high level production of terpenoids in *E. coli*[38]. The components of the heterologous MVA pathway have been introduced into *E. coli* genomes for increase the supply of IPP and DMAPP[39]. Till date, *B. subtilis168* and *Bacillus subtilis natto* has been identified as attractive hosts for the production of Vitamin K2(Figure.7c, g). De novo biosynthesis of Menaquinone-7 from glycerol in the metabolic engineered *Bacillus subtilis 168*, the MK-7 production had insignificant increase to 69.5 ± 2.8 mg/L upon 144 h fermentation in a 2 L baffled flask[34]. In addition, Berenjian et al. further increased menaquinone-7 concentration of 226 mg/L at 1,000 rpm, 5 vvm, 40 degrees C after 5 days of fermentation[40]. MK7 can exist as geometric isomers that can occur in the *cis, trans*, and *cis/trans* forms; however, only the all-*trans* from is biologically significant[41]. Those result is in accord with the fact of heptaprenyl diphosphate synthase(convert Famesyl-PP to Heptaprenyl- PP) were identified in *Bacillus subtilis* genomic DNA.

It is important to mention that *Streptomyces* possessed the futalosine pathway for menaquinone biosynthesis(Fig. 7d, S10c), encompassing seven enzymes encoded by the Mqn gene cluster[42]. Our Figure also show that the geranylgeranyl diphosphate synthase(four isoprene units) and heptaprenyl diphosphate synthase(seven isoprene units) are found in *Streptomyces* genomes, which is not consistent with previous studies have shown that *Streptomyce* possess MK-9 as the major menaquinone component[43]. It was believed that *Streptomyces* possessed a mevalonate pathway or MEP pathways in different species[44–47]. This issues of *Nocard's bacillus* and *Mycobacterium tuberculosis* were few previously described in detail. In *Mycobacterium* strains, the predominant menaquinones had nine isoprenoid units with one double bond hydrogenated (dihydromenaquinones), abbreviated as MK-9 while *Nocardia* strains predominantly contained tetrahydromenaquinones MK-8[48]. Interestingly, it must be noted that *Nocard's bacillus* possessed MVA pathway and MEP pathway at same time in one strain(Figure.7e).

As list in Table 4, the analysis of MKn among different strains reveal that almost every strain produces multiple types of MK and a certain type of MK accounts for the predominant proportion[20, 43, 49–52]. In addition, a confusing phenomenon were observed in view of the above analysis: theoretical types of MK_n were not consistent with experimental values. Hence, the relationship between the length of side chain and the type of diphosphate synthase still need to be explored in detail.

Table 4
Mennaquinone composition of various strains.

Strains	Menaquinone present	Major component	Reference
Elizabethkingia	MK_4, MK_5, MK_6	MK_6	W Wei et al. (2018)
Meningoseptica	MK_8, MK_9, MK_{10}	MK_9	Collins et al. (1984)
Streptomyces	MK_6, MK_7, MK_8	MK_8	Collins et al. (1977)
Nocard's bacillus	MK_7, MK_8, MK_9	MK_9	Pa P Patrick et al. (1971)
Mycobacterium	MK_6, MK_7	MK_7	SATO et al. (1971)
tuberculosis	MK_4,MK_6,MK_7,MK_8,MK_9	MK_9	Magali et al. (2017)
Bacillus subtilis natto			
Lactococcus			

Conclusion

In this study, the disclosure of the genome sequences of *Elizabethkingia meningoseptica sp. F2* improves our understanding on the evolutionary relationship of *Elizabethkingia* and Vitamin K2 metabolic pathway. Comparative analysis of *Elizabethkingia meningoseptica sp. F2*, *Elizabethkingia meningoseptica sp.NBRC12535* and *Elizabethkingia meningoseptica sp. ATCC13253* revealed highly similar with each other. Detailed and systematic analysis on different isoprene side chain biosynthesis pathway relevant genes provides deep insights on the relationship between the length of side chain and the type of diphosphate synthase through the compare with other strains. There are multiple antibiotics resistance genes in genome, antibiotic sensitivity data for *Elizabethkingia meningoseptica sp. F2* is crucial for guiding further research. Prenyltransferase MenA is a vital class involved in the biosynthesis of Vitamin K2. In conclusion, the current study shows potential by providing the whole genomic Sequence and Vitamin K2 metabolic pathway as basis material for the functional expression of key gene in Vitamin K2 biosynthesis pathway.

Declarations

Author Contribution Statement

ZZM, ZGH and YQ conceived and designed research. YQ conducted experiments. LH, WP and WL contributed new reagents or analytical tools. SXW, NWF, WH and THF analyzed data. YQ wrote the manuscript. All authors read and approved the manuscript.

Funding

This research was funded by the Key 863 Fund of China (2014AA021704), Major Projects of Science and Technology in Anhui Province (17030801036), Key research and development plan of Anhui Province

(1804b06020342) and Natural Science Foundation of Anhui Province (1908085MB48, 1608085QC46).

Availability of data and materials

All data supporting the conclusions of this study are included in this article and the additional file.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1 Key Laboratory of High Magnetic Field and Ion Beam Physical Biology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei 230031,Anhui, People's Republic of China.

2 University of Science and Technology of China, Hefei 230026, Anhui, People's Republic of China.

References

1. Schurgers LJ, Knapen MHJ, Vermeer C. **Vitamin K2 improves bone strength in postmenopausal women**. 2007, 1297:0–187.
2. Vermeer C, Schurgers LJ. A COMPREHENSIVE REVIEW OF VITAMIN K AND VITAMIN K ANTAGONISTS. Hematol Oncol Clin North Am. 2000;14:339–53.
3. Wen L, Jiepeng C, Lili D, Shuzhuang L. **Vitamin K?dependent proteins involved in bone and cardiovascular health (Review)**. *Molecular Medicine Reports* 2018.
4. Bhalerao S, Clandinin TR. Vitamin K2 Takes Charge. Science. 2012;336:1241–2.
5. Michio K, Eeshwaraiah B. **Vitamin K2 in Electron Transport System: Are Enzymes Involved in Vitamin K2 Biosynthesis Promising Drug Targets?** *Molecules* 2010, 15:1531–1553.
6. Vermeer C, Vitamin K. The effect on health beyond coagulation-an overview. Food Nutr Res. 2012;56:5329–34.
7. Walther B, Karl JP, Booth SL, Boyaval P. Menaquinones, Bacteria, and the Food Supply: The Relevance of Dairy and Fermented Food Products to Vitamin K Requirements. Advances in Nutrition. 2013;4:463–73.

8. Jean SS, Hsieh TC, Ning YZ, Hsueh PR. **Role of vancomycin in the treatment of bacteraemia and meningitis caused by Elizabethkingia meningoseptica**. *Int J Antimicrob Ag* 2017, 50.
9. Kim KK. Transfer of Chryseobacterium meningosepticum and Chryseobacterium miricola to Elizabethkingia gen. nov. as Elizabethkingia meningoseptica comb. nov. and Elizabethkingia miricola comb. nov. Int J Syst Evol Microbiol. 2005;55:1287–93.
10. Jean SS, Lee WS, Chen FL, Ou TY, Hsueh PR. Elizabethkingia meningoseptica: an important emerging pathogen causing healthcare-associated infections. J Hosp Infect. 2014;86:244–9.
11. Hung PP, Lin YH, Lin CF, Liu MF, Shi ZY: **Chryseobacterium meningosepticum infection**: **Antibiotic susceptibility and risk factors for mortality**. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi* 2008, **41**:137–144.
12. Bolash NKLH. Quinolone susceptibility of multi resistant Flavobacterium meningosepticum clinical isolates in one urban hospital. Drugs. 1995;49:3.
13. Tani Y, Sakurai N. Menaquinone-4 Production by a Mutant of Flavobacterium sp. 238-7. Agricultural Biological Chemistry. 1987;51:2409–15.
14. Taguchi H, Shibata T, Duangmanee C, Tani Y. Menaquinone-4 Production by a Sulfonamide-resistant Mutant of Flavobacterium sp. 238–7. Agric Biol Chem. 1989;53:3017–23.
15. Tagvchi H, Shibata T, Tani Y. Selective Release of Menaquinone-4 from Cells of a Mutant of Flavobacterium sp. 238–7 with a Detergent. Bioscience Biotechnology Biochemistry. 1995;59:1137–8.
16. Tani Y, Yamada SA,H. Vitamin K 2 (menaquinone): screening of producing microorganism and production by Flavobacterium meningosepticum. J Ferment Technol. 1984;62:7.
17. Boock JT, Gupta A, Prather KL. Screening and modular design for metabolic pathway optimization. Curr Opin Biotech. 2015;36:189–98.
18. Liu CL, Dong HG, Zhan J, Liu X, Yang Y. Multi-modular engineering for renewable production of isoprene via mevalonate pathway in Escherichia coli. J Appl Microbiol. 2019;126:12.
19. Fang XYQ, Liu H, Wang P, Wang L, Zheng ZZG. **Effects of a combined processing technology involving ultrasound and surfactant on the metabolic synthesis of vitamin K2 by** *Flavobacterium* **sp. M1-14**,. Chemical Engineering Processing - Process Intensification. 2018;135:9.
20. Wei H, Wang L, Zhao G, Fang Z, Wu H, Wang P, Zheng Z. Extraction, purification and identification of menaquinones from Flavobacterium meningosepticum fermentation medium. Process Biochem. 2018;66:245–53.
21. Lowe T, Eddy S. **tRNAscan-SE: A program for improved detection of transfer RNA Genes in genomic sequence**. *Nucleic Acids Res* 1997, 25.
22. Steinberg JP, Burd EM. **238 – Other Gram-Negative and Gram-Variable Bacilli**. 2015:2667–2683.
23. Rossolini G, Franceschini N, Riccio M, Mercuri P, Perilli M, Galleni M, Frère J-M, Amicosante G. Characterization and sequence of the Chryseobacterium (Flavobacterium) meningosepticum

carbapenemase: A new molecular class B β-lactamase showing a broad substrate profile. Biochem J. 1998;332(Pt 1):145–52.

24. Ratner H. Flavobacterium meningosepticum. Infection Control Ic. 1984;5:237–9.
25. Bauer AW, Kirby WMM, Sherris JCA, Turck M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. Technical bulletin of the Registry of Medical Technologists. 1966;36:49–52.
26. King E. Studies on a Group of Previously Unclassified Bacteria Associated with Meningitis in Infants. Am J Clin Pathol. 1959;31:241–7.
27. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. Circos: An information aesthetic for comparative genomics. Genome Res. 2009;19:1639–45.
28. Coenye T. Extracting phylogenetic information from whole-genome sequencing projects: the lactic acid bacteria as a test case. Microbiology +. 2003;149:3507–17.
29. Woese CR, Kandler OML, Wheelis ML. Towards a Natural System of Organisms - Proposal for the Domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A. 1990;87:4576–9.
30. Lassalle F, Périan S, Bataillon T, Nesme X, Duret L, Daubin V, Petrov DA. GC-Content Evolution in Bacterial Genomes: The Biased Gene Conversion Hypothesis Expands. Plos Genetics. 2015;11:e1004941.
31. Markus Lange B, Rujan T, Martin W, Croteau R. **Isoprenoid biosynthesis: The evolution of two ancient and distinct pathways across genomes**. 2000, 97:13172–13177.
32. Wei H, Zhao G, Liu H, Wang H, Ni W, Wang P, Zheng Z. **A simple and efficient method for the extraction and separation of menaquinone homologs from wet biomass of Flavobacterium**. *Bioprocess & Biosystems Engineering* 2017.
33. Xu JZ, Yan W-L, Zhang W-G. Enhancing menaquinone-7 production in recombinant Bacillus amyloliquefaciens by metabolic pathway engineering. Rsc Advances. 2017;7:28527–34.
34. **Modular Pathway Engineering of Bacillus subtilis To Promote De Novo Biosynthesis of Menaquinone7**. *ACS Synthetic Biology* 2019, **8**:12.
35. Min KK, Lee PC. **Metabolic engineering of menaquinone-8 pathway of Escherichia coli as a microbial platform for vitamin K production**. 2011, 108:1997–2002.
36. Bentley R, Meganathan R. **Biosynthesis of the isoprenoid quinones ubiquinone and menaquinone**. *Escherichia Coli and Salmonella Typhimurium* 1987:512–520.
37. Kawamukai M. **Biosynthesis and applications of prenylquinones**. *Biosci Biotechnol Biochem* 2018:1–15.
38. Martin VJJ, Pitera DJ, Withers ST, Newman JD, Keasling JD. Engineering a mevalonate pathway in Escherichia coli for production of terpenoids. Nat Biotechnol. 2003;21:796–802.
39. Zong Z, Hua Q, Tong X, Li D, Wang C, Guo D, Liu Z. Biosynthesis of nerol from glucose in the metabolic engineered Escherichia coli. Bioresource Technol. 2019;287:121410.
40. Berenjian A, Mahanama R, Talbot A, Regtop H, Kavanagh J, Dehghani F. Designing of an Intensification Process for Biosynthesis and Recovery of Menaquinone-7. Applied Biochemistry

Biotechnology. 2014;172:1347–57.

41. Lal N, Berenjian A. **Cis and trans isomers of the vitamin menaquinone-7: which one is biologically significant?** *Appl Microbiol Biot* 2020.
42. Hiratsuka T, Furihata K, Ishikawa J, Yamashita H, Itoh N, Seto H, Dairi T. An Alternative Menaquinone Biosynthetic Pathway Operating in Microorganisms. Seikagaku. 2008;321:1670–3.
43. Collins MD, Faulkner M, Keddie RM. Menaquinone Composition of Some Sporeforming Actinomycetes. Systematic Applied Microbiology. 1984;5:20–9.
44. Takagi M, Kuzuyama T, Takahashi S, Seto H. A Gene Cluster for the Mevalonate Pathway from Streptomyces sp. Strain CL190. J Bacteriol. 2000;182:4153–7.
45. Shin-Ya K, Furihata K, Hayakawa Y, Seto H. Biosynthetic studies of naphterpin, a terpenoid metabolite of Streptomyces. Tetrahedron Lett. 1990;31:6025–6.
46. Kuzuyama T. **Biosynthetic studies on terpenoids produced by Streptomyces**. *J Antibiot* 2017.
47. Cane DE, Chow C, Lillo A, Kang I. Molecular cloning, expression and characterization of the first three genes in the mevalonate-independent isoprenoid pathway in Streptomyces coelicolor. Bioorgan Med Chem. 2001;9:1467–77.
48. Minnikin DE. Distribution of Menaquinones in Actinomycetes and Corynebacteria. J Gen Microbiol. 1977;100:221–30.
49. Walther B. **Determination of menaquinone production by Lactococcus spp. and propionibacteria in cheese**. *Int Dairy J* 2017:S0958694617301541.
50. Araki. **Production of Menaquinone (vitamin K2)-7 by Bacillus subtilis**. *J Biosci Bioeng*.
51. Collins MD, Pirouz T, Goodfellow M, Minnikin DE. Distribution of menaquinones in actinomycetes and corynebacteria. J Gen Microbiol. 1977;100:221–30.
52. DUNPHY PATRICKJ, PGP ARNOLDFBRODIE. Separation and identification of menaquinones irom microorganisms. JOURNAL OF LIPID RESEAR. 1971;12:8.

Figures

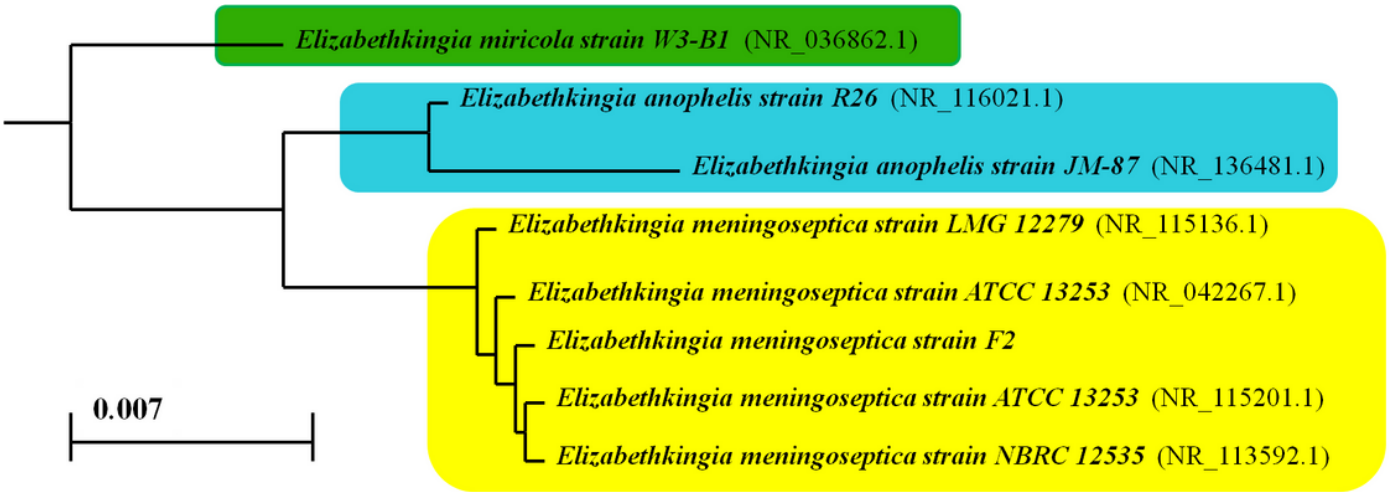

Figure 1

Phylogenetic relationship among the Elizabethkingia species strains based on 16S rRNA gene sequences. The Phylogenetic tree was made with NCBI BLAST pairwise alignments using the Fast Minimum Evolution method.

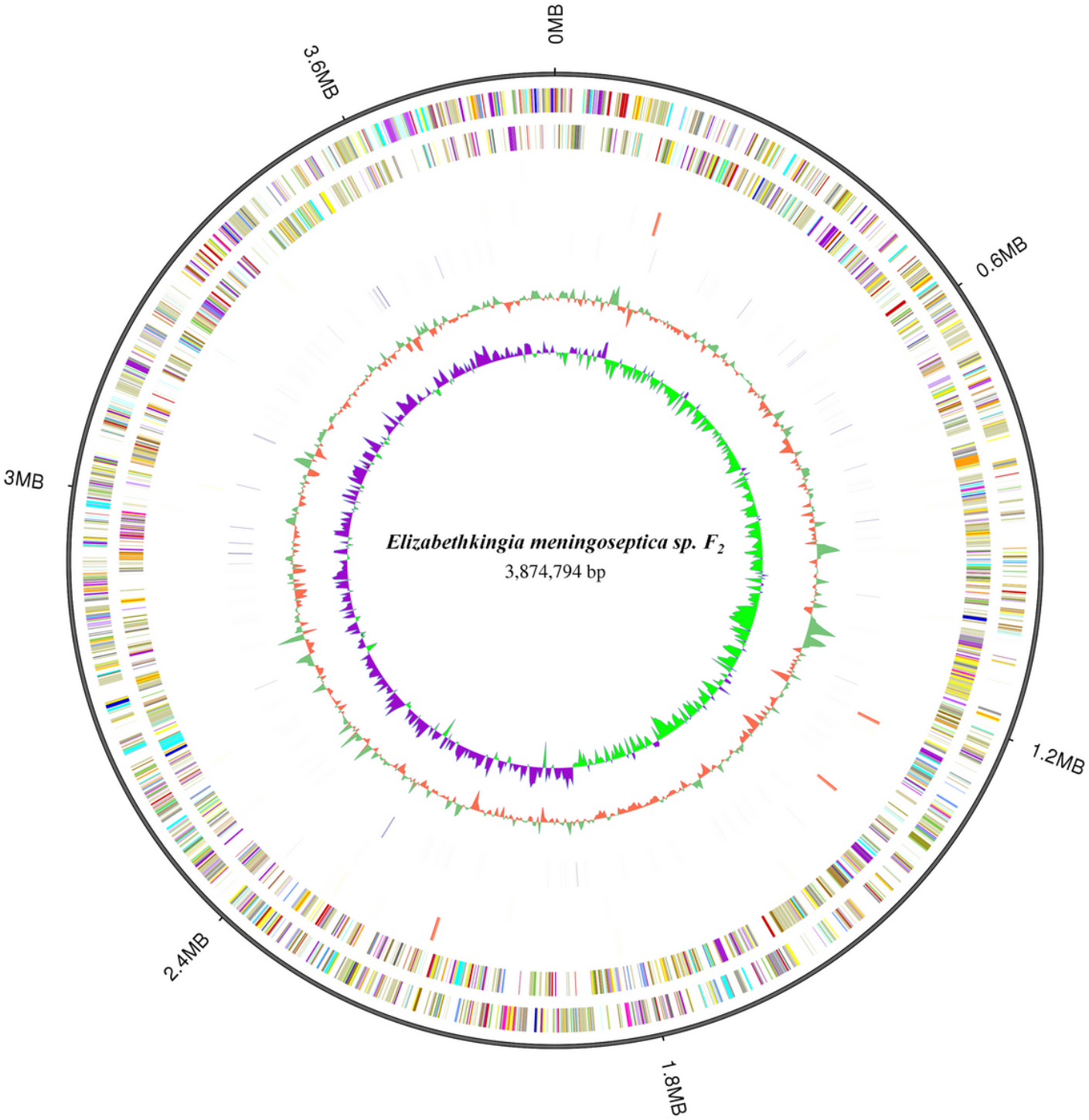

Figure 2

A circular representation of the Elizabethkingia meningoseptica sp. F2 chromosome illustrating the location of each predicted protein-coding region as well as selected features of the genome, colored according to cluster of orthologous groups (COG)classification. From outer to inner : 1. Genome Size 2. Forward Strand Gene, colored according to cluster of orthologous groups (COG) classification 3. Reverse Strand Gene, colored according to cluster of orthologous groups (COG) classification 4. Forward Strand ncRNA 5. Reverse Strand ncRNA 6. Repeat 7. GC 8. GC-SKEW

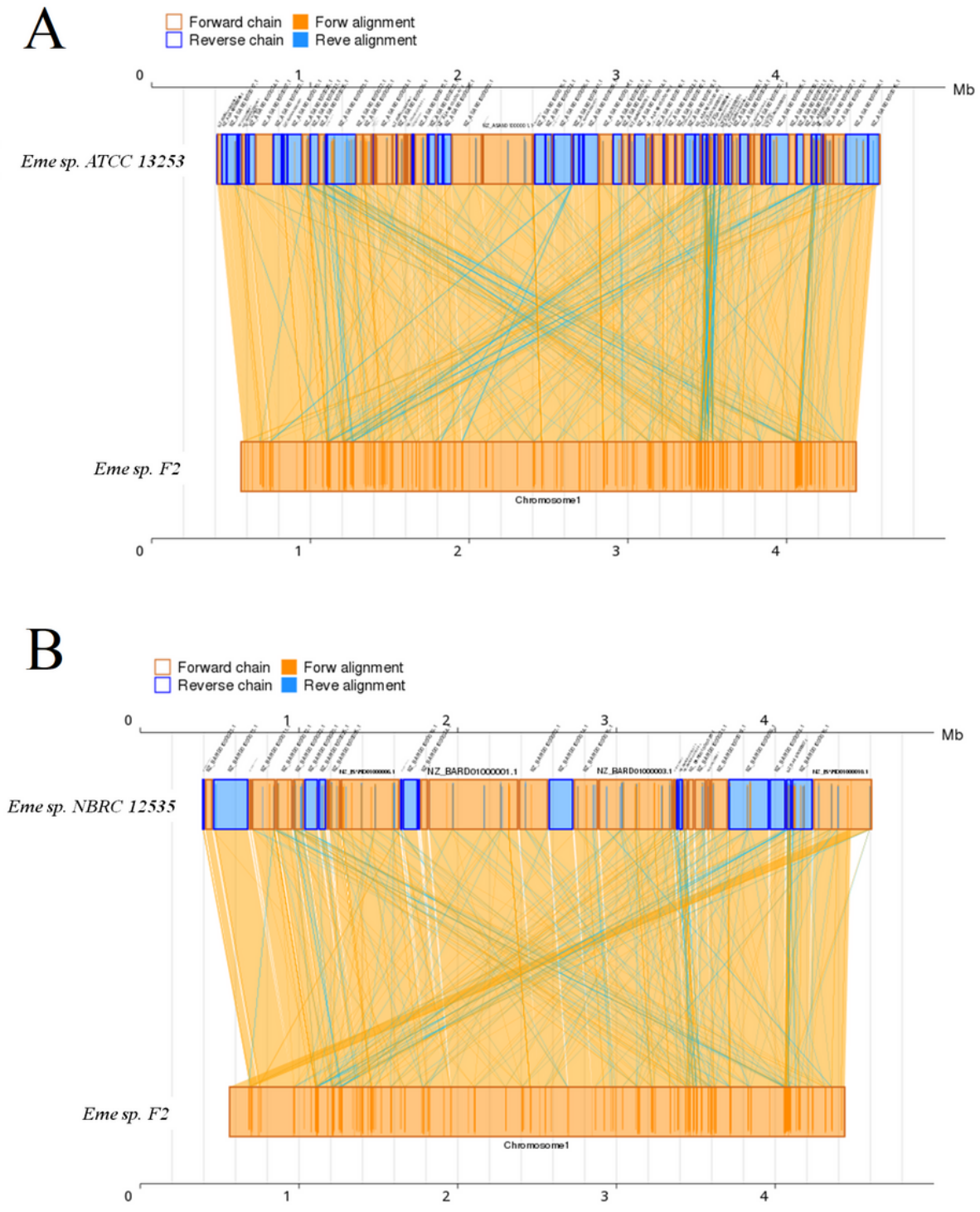

Figure 3

Elizabethkingia meningoseptica sp. F2(Eme) , Elizabethkingia meningoseptica sp.ATCC13253, Elizabethkingia meningoseptica sp.NBRC12535 nucleic acid level synteny.Yellow box stands for forward chain and blue box stands for reverse chain within the upper and following sequence region. In the box of sequence, the yellow region stands for the nucleic acid sequence in the forward chain of this genome sequence and the blue region stands for the nucleic acid sequence in the reverse chain of this genome sequence. In the middle region of two sequences, the yellow line stands for forward alignment and the blue line stands for reverse complementary alignment.

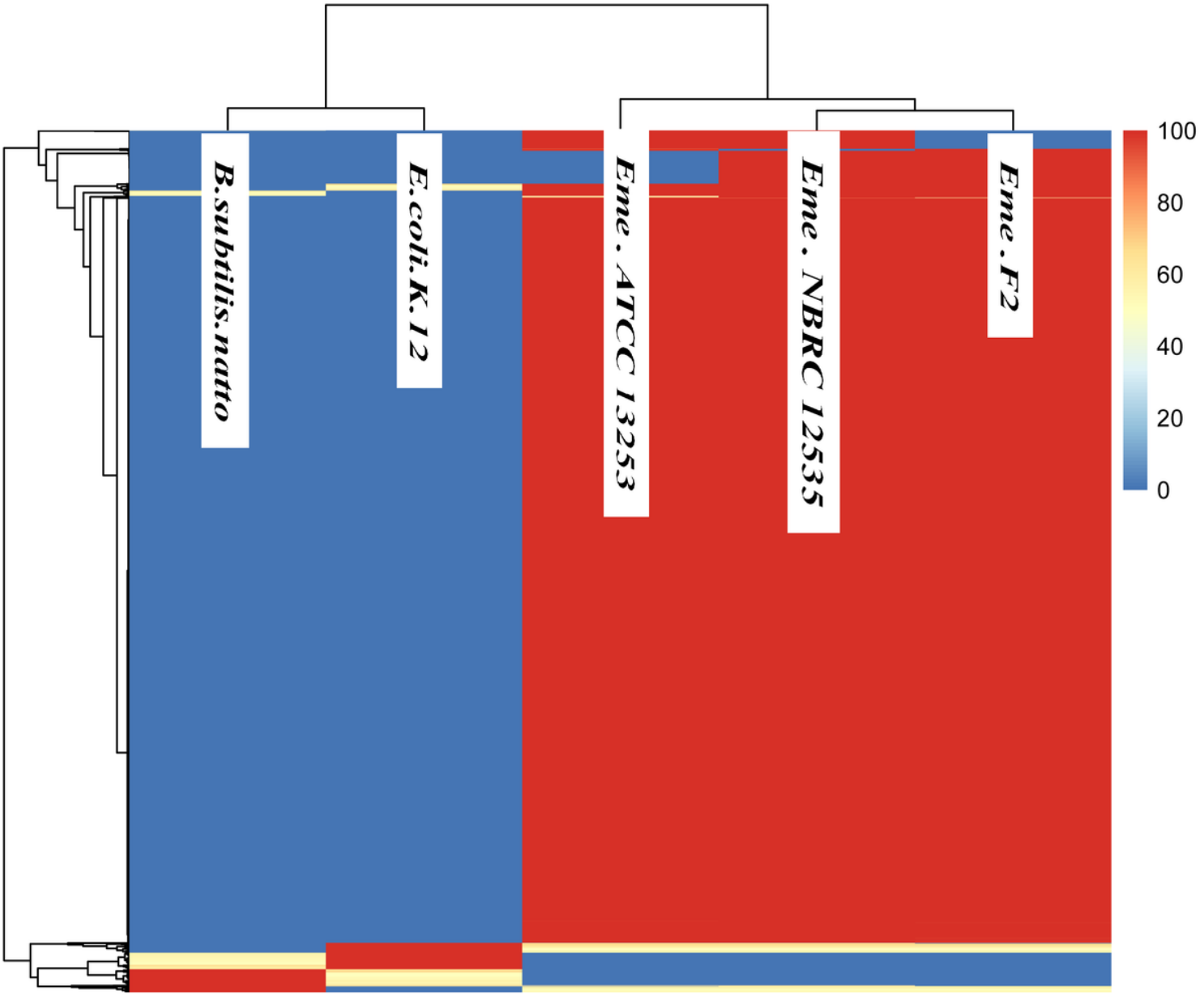

Figure 4

Dispensable gene heat map. below are each strain name, left are Dispensable gene cluster, top are strain cluster, the similarities of gene are shown in the middle with different color represent different coverage by heat map. color/depth in top left pic.

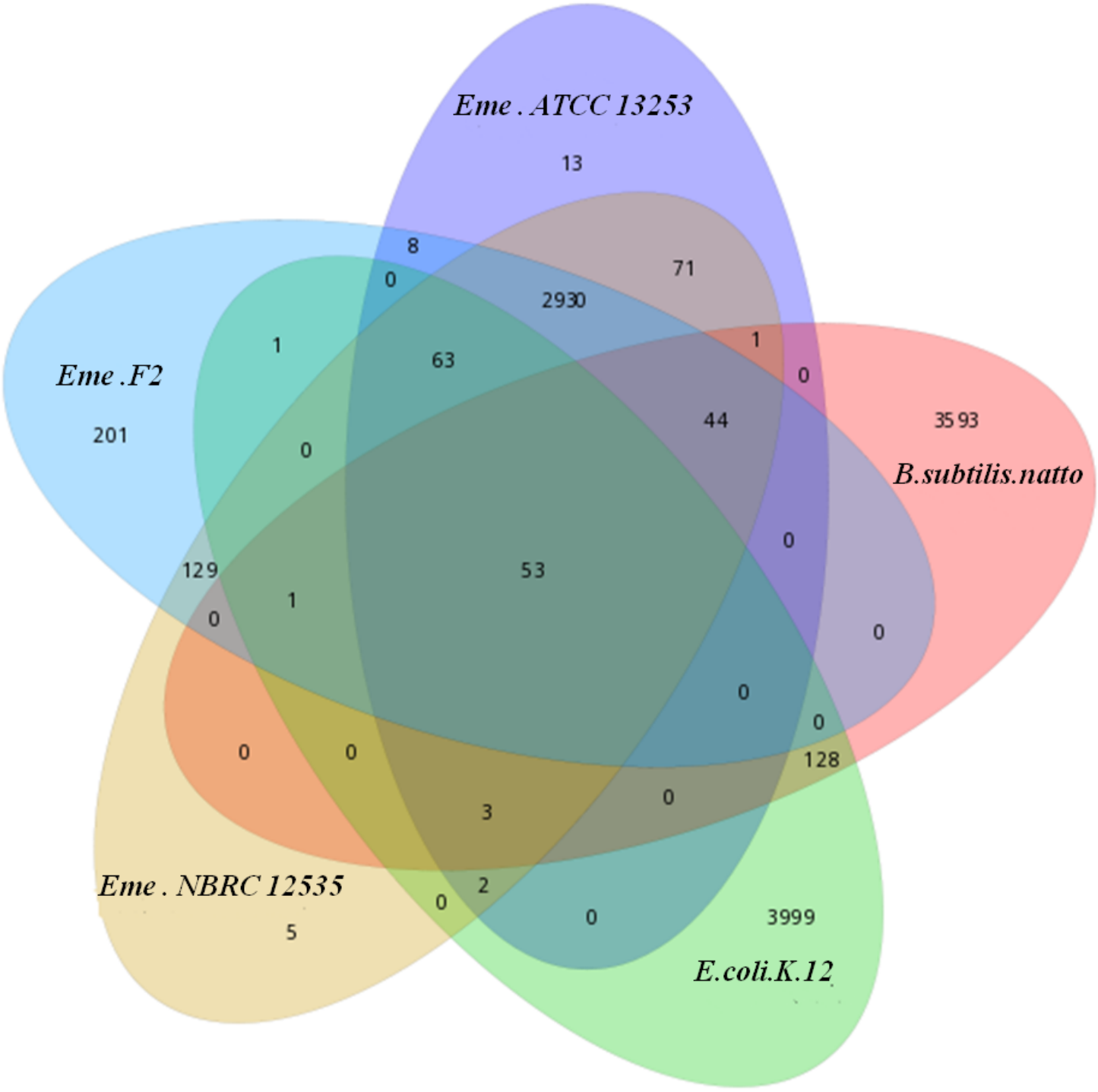

Figure 5

Venn graph of Pan gene.Each ellipse represent one strain, the number in the ellipse means the only cluster number. One cluster have the genes that more than 50 percent identity and less than 0.3 length diversity.

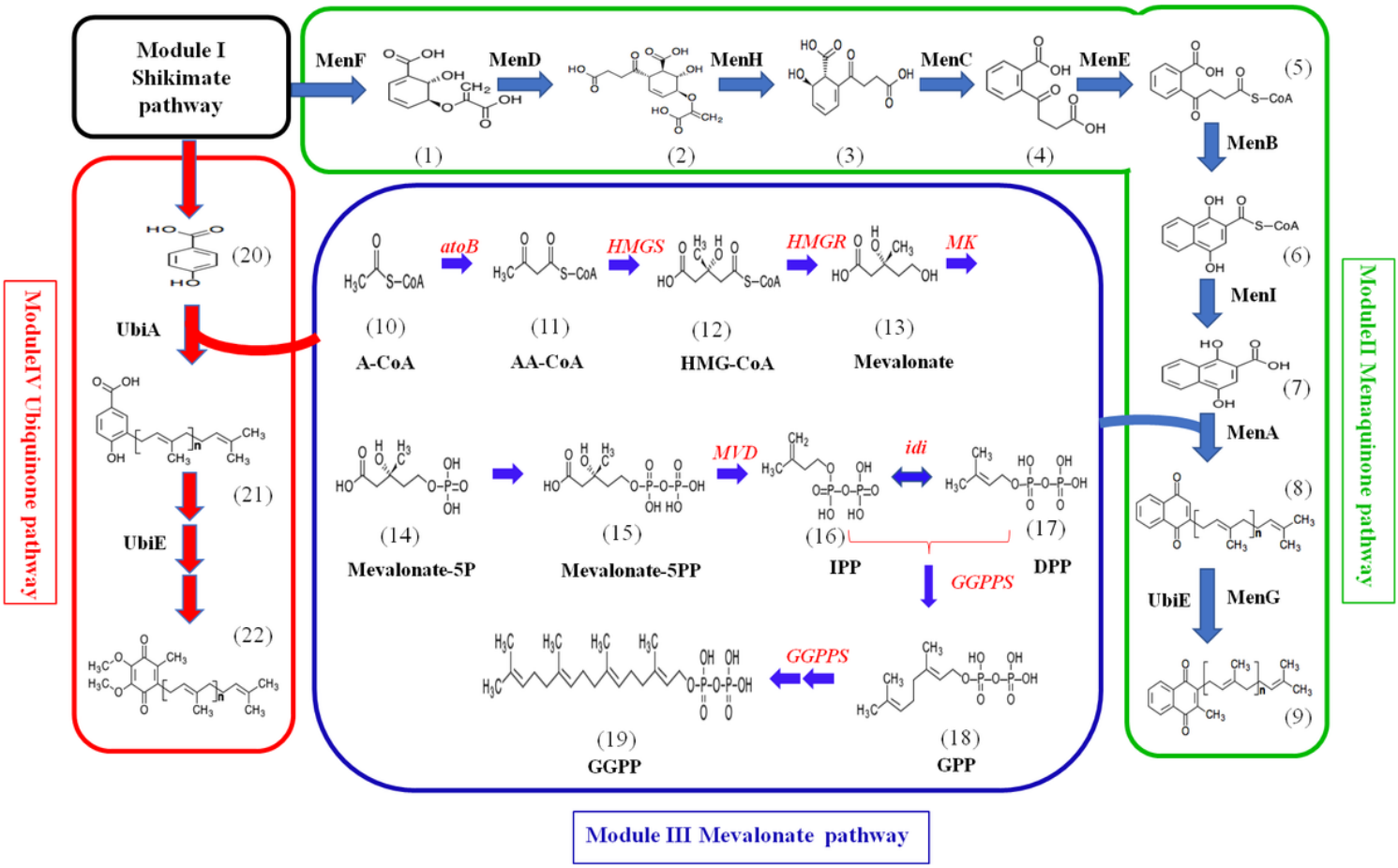

Figure 6

Schematic diagram of the menaquinone and ubiquinone biosynthetic pathway in Elizabethkingia meningoseptica sp. F2, which was categorized into three modules I–IV. Module I: Shikimate pathway. Module II: Menaquinone pathway: (1) Isochorismate, (2) 2-Succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1- carboxylate,(3) (1R,6R)-6-Hydroxy-2- succinylcyclohexa-2,4-diene-1-carboxylate, (4) 2-Succinylbenzoate, (5) Succinylbenzoyl-CoA, (6)1,4-Dihydroxy-2- naphthoyl-CoA,(7)1,4-Dihydroxy-2-naphthoate, (8) 2-Demethylmenaquinone, (9) Menaquinone. Module III: Mevalonate pathway: (10) Acetyl-CoA (11) Acetoacetyl-CoA (12) 3-hydroxy-3-methylglutaryl-CoA (13) Mevalonate (14) Mevalonate-5P (15) Mevalonate-5PP (16) Isopentenyl diphosphate (17) Dimethylallyl diphosphate (18) Geranyl pyrophosphate (19) Geranylgeranyl diphosphate. Module IV: Ubiquinone pathway: (20) 4-Hydroxybenzoate, (21) 4-Hydroxy-3- polyprenylbenzoate, (22) Ubiquinone.

Figure 7

The metabolic pathway of menaquinone biosynthesis in different strains. Classic menaquinone pathway: MenF (menaquinone-specific isochorismate synthase), MenD(2-succinyl-6-hydroxy-2,4- cyclohexadiene-1-carboxylate synthase/2-oxoglutarate decarboxylase), MenH(2-succinyl-6-hydroxy-2,4- cyclohexadiene-1-carboxylate synthase), MenC(osuccinylbenzoate-CoA synthase), MenE(o-succinylbenzoic acid-CoA ligase), MenB(naphthoate synthase), MenI(1,4-dihydroxy-2-naphthoyl-CoA hydrolase), MenA (1,4-dihydroxy-2-naphthoate octaprenyltransferase), MenG and UbiE(Q-8/MK-8 biosynthesis methyltransferase) Futalosine pathway: MqnA, chorismate dehydratase, MqnE, aminodeoxyfutalosine synthase, MqnB futalosine hydrolase, MqnC, cyclic dehypoxanthinyl futalosine synthase, MqnD 1,4-dihydroxy-6-naphtoate synthase. Mevalonate pathway:A mevalonate pathway: atoB, acetoacetyl-CoA acetyltransferase; HMGS, HMG-CoA synthase; HMGR, HMG-CoA reductase; MK, mevalonate kinase; MVD, mevalonate pyrophosphate decarboxylase; idi, IPP isomerase. MEP pathway: Dxs, 1-deoxyxylulose-5-phosphate synthase, Dxr,1-deoxy-D-xylulose 5-phosphate reductoisomerase, ispD ,4-diphosphocytidyl-2C-methyl-D-erythritol synthase, ispE 4-diphosphocytidyl-2-C-methylerythritol kinase, ispF 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, ispG 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase, ispH, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, idi, isopentenyl diphosphate isomerase, ispA, geranyltranstransferase, ispB,octaprenyl diphosphate synthase.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docxLAST.docx