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ORIGINAL ARTICLE

Genomic insight into *Enterococcus faecium* biofilm formation isolated from catheter associated urinary tract infection

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ABSTRACT

Background and Objectives: *Enterococcus faecium* is a major nosocomial pathogen frequently associated with catheter-related infections and biofilm-mediated antimicrobial resistance. This study aimed to investigate the genomic determinants and structural characteristics underlying biofilm formation in a clinical *E. faecium* isolate recovered from a catheter-associated urinary tract infection (CAUTI).

Materials and Methods: A clinical isolate, *E. faecium* MFKCC-BF.UR33, was subjected to whole-genome sequencing and comprehensive genome annotation to identify biofilm-associated and antimicrobial resistance genes. Phylogenetic analysis was performed to determine its relatedness to reference clinical strains. Structural modeling of selected biofilm-associated proteins was conducted using AlphaFold2 to assess their three-dimensional conformations and potential as therapeutic targets.

Results: Whole-genome sequencing revealed a 3.32 Mb genome with a G+C content of 37.35%, comprising 2,321 predicted coding sequences and no detectable plasmids. Genome annotation identified several biofilm-associated genes, including *sgrA*, *acm*, *fss3*, *scm*, and *efbA*, as well as antimicrobial resistance genes (*antA/B*, *tetM*, *tetL*, *efmA*, and *liaFSR*). Phylogenetic

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analysis placed the isolate within the *E. faecium* clade, closely related to other clinical reference strains. Structural modeling demonstrated high-confidence 3D structures for Fss3 and EfbA proteins.

Conclusion: The integration of genomic and structural analyses provides insights into the genetic and structural basis of biofilm formation in *E. faecium* and establishes a foundation for future functional and structure-based investigations.

Keywords: *Enterococcus faecium*; Biofilms; Drug resistance; Microbial; Genome; Bacterial; Catheter-related infections

INTRODUCTION

Bacterial biofilms play a critical role in the pathogenesis of infectious diseases, marking their formation as a complex and clinically relevant research area. Biofilm formation is a complex, multistage process in which microorganisms transition from a free-living planktonic state to a structured community attached to biotic or abiotic surfaces and embedded within a self-produced extracellular polymeric substance (EPS) matrix. The process generally consists of initial attachment, irreversible adhesion, microcolony formation, biofilm maturation, and eventual dispersal. The EPS matrix provides structural stability and protection against environmental stressors, host immune responses, and antimicrobial agents. Although this process is conserved among many microbial species, the regulatory pathways, signaling mechanisms, and environmental triggers involved may vary depending on the organism and surrounding conditions (1, 2). Biofilms enhance the virulence and persistence of many pathogens. Recent studies estimate that they contribute to 60-80% of microbial infections, underscoring their significant impact on human health (3). These structured microbial communities commonly colonize medical devices such as catheters, prostheses, and heart valves, as well as various epithelial surfaces. In clinical settings, biofilm-associated infections pose a major challenge due to their high resistance to antibiotics, which often leads to chronic inflammation and complicates therapeutic management (4).

Enterococcus faecium is a major nosocomial pathogen that is commonly transmitted in healthcare settings through contaminated healthcare workers' hands, shared medical equipment, environmental surfaces, and direct or indirect patient-to-patient contact. Its ability to survive for prolonged periods on hospital surfaces and medical devices facilitates its dissemination within healthcare facilities. Furthermore, the widespread use of invasive devices, prolonged hospitalization, and antimicrobial exposure contribute to the successful transmission and persistence of *E. faecium*, particularly in intensive care units and other high-risk clinical settings (5). *E. faecium* is a well-established pathogen and known for its capacity to form robust biofilms, which significantly enhance its virulence and persistence in clinical settings. Biofilm formation on medical devices, particularly catheters, has been associated with severe, persistent infections, increased morbidity, and higher mortality rates (6). Urine-grown *Enterococcus* cells exhibit changes in surface properties, such as reduced hydrophobicity and altered surface energy, which may enhance their adhesion to urinary tract surfaces or catheters (7). The ability of *E. faecium* to form biofilm plays a central role in their pathogenicity, and therefore increase their prevalence of nosocomial infections (8). Studies emphasized the clinical relevance of *E. faecium* biofilms, particularly in the context of hospital-acquired infections and the difficulties in achieving effective eradication (9, 10). Notably, certain strains demonstrate a remarkable capacity to form resilient biofilms, further complicating infection control and treatment efforts (10).

Understanding the genetic mechanisms underlying biofilm formation in *E. faecium* through whole genome sequencing (WGS) is essential for elucidating its pathogenic strategies and for identifying novel therapeutic targets against biofilm-associated infections. Biofilm development in *Enterococcus* is driven by a complex interplay of genes and virulence factors, including gelatinase, cytolysin, and secreted antigen A, which facilitate bacterial adherence and colonization (9). These factors contribute to infection persistence by enhancing biofilm resistance to both host immune defenses and antimicrobial agents. Additionally, biofilm production is tightly regulated by genetic signaling systems such as quorum sensing, adding another layer of complexity to its regulation. Several adhesin-encoding genes and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), including Acm, Scm, Fss3, and SgrA, have been implicated in *Enterococcus* biofilm formation, highlighting the intricate genetic basis of this phenotype and its relevance in clinical

contexts (9, 11).

Enterococci exhibit a high level of intrinsic resistance to several antimicrobial agents, including cephalosporins, clindamycin, and aminoglycosides. Among them, *E. faecium* is often more resistant than other species, as reflected by the higher prevalence of glycopeptide-resistant and vancomycin-resistant *E. faecium* compared to *E. faecalis* (12). Similarly, a study by (13) reported high levels of resistance in *E. faecium*, with 92.5% of isolates resistant to ampicillin, 96% to ciprofloxacin, 100% to erythromycin, and 96% to clindamycin. The antibiotic resistance observed in *E. faecium* is driven by a combination of intrinsic traits, biofilm formation, and acquired mechanisms such as horizontal gene transfer and target-site modifications. These factors, coupled with its genomic plasticity, make *E. faecium* a formidable nosocomial pathogen (14, 15).

E. faecium has been shown to form biofilms both in vitro and in vivo on indwelling medical devices, particularly urinary catheters. The pathophysiology of catheter-associated urinary tract infections (CAUTIs) and biofilm development is strongly influenced by virulence factors such as adherence proteins and surface-associated antigens, including Esp and Ebp. The adhesion factor plays a crucial role in the aetiology of CAUTIs, as *E. faecium* exhibits stronger adherence to catheter surfaces compared to other *Enterococcus* species. Surface proteins Esp and Ebp further promote colonization by binding to both catheter materials and epithelial tissues, thereby facilitating robust biofilm formation (16).

The management of CAUTIs caused by biofilm-forming *E. faecium* involves several strategies, including prompt replacement of the urinary catheter, implementation of CAUTI prevention bundles, rational antimicrobial prescribing, selection of appropriate catheter materials (e.g., silicone, which supports less biofilm formation than latex), and exploration of novel coatings such as antimicrobial peptides or silver nanoparticles. Antibiotics such as nitrofurantoin, amoxicillin, ampicillin, or levofloxacin may be used to treat susceptible strains. Ampicillin-resistant *Enterococcus* can be treated with vancomycin or linezolid, while strains resistant to both ampicillin and vancomycin may be treated with linezolid or daptomycin. The recommended duration of antibiotic therapy is 7 days for cases with rapid symptom resolution and 10-14 days for those with a delayed clinical response (17, 18).

This study focused on sequencing the complete genome of an *E. faecium* isolate to enable an in-depth investigation of the genetic factors involved in biofilm formation. The resulting data aim to enhance our understanding of the molecular determinants underlying biofilm-associated infections in *E. faecium*, with the ultimate objective of identifying novel therapeutic targets to combat these persistent, biofilm-mediated infections.

MATERIALS AND METHODS

Sample preparation and isolate identification. A clinical isolate of MFKCC-BF.UR33 was obtained from the archived bacterial collection of the Microbiology Laboratory, Faculty of Medicine, Universitas Gadjah Mada. The isolate originated from a urine specimen of a catheterized patient diagnosed with CAUTI in the Intensive Care Unit and was selected for this study based on its biofilm-forming phenotype identified in a previous investigation. The urine culture showed significant growth of infection-causing microorganisms, with no more than two species present and bacterial counts reaching $\geq 10^5$ CFU/mL. The isolate was re-cultured on blood agar and incubated for 18-24 h prior to analysis. Species identification and antimicrobial susceptibility testing were performed using the VITEK 2 Compact system. The isolate was selected for whole-genome sequencing based on its strong biofilm-forming phenotype previously determined using the Microtiter Plate Assay (MTPA) as part of an ongoing study.

Whole-genome sequencing. Genomic DNA was extracted from the isolate that were incubated overnight at 37°C on blood agar medium (Oxoid), following the protocols of the Quick-DNA MagBead Kit (Zymo Research, D4081). The DNA concentration was quantified using a Qubit 3.0 Fluorometer (Life Technologies, Frederick, MD, USA) in combination with the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). Sequencing libraries were prepared using library preparation kits from Oxford Nanopore Technologies. The libraries were then sequenced on the GridION platform, operated with MinKNOW software (version 23.04.5).

Sequence assembly and genome analysis. The quality of raw reads was assessed using NanoPlot.

Reads were filtered with Filtlong and assembled de novo using Flye (v2.8.3). Genome mapping and polishing were performed through four rounds of polishing using Minimap2 (v2.24-r1122) and Racon (v1.5.0), followed by three rounds using Medaka (v1.7.2). Closely related species and genome completeness were evaluated using DFAST-qc (v0.4.2) and BUSCO (v5.4.4), respectively. Assembly quality was further assessed using QUAST (v5.0.2). Genome annotation and visualization was performed with Prokka (https://proksee.ca/).

Phylogenetic tree analysis. Whole-genome assemblies were submitted to the Type (Strain) Genome Server (TYGS; https://tygs.dsmz.de) to generate a genome-based phylogenetic tree. TYGS performed pairwise comparisons and calculated intergenomic distances using the Genome BLAST Distance Phylogeny (GBDP) method under default parameters. The resulting phylogenetic tree file (.phy) was downloaded and further visualized using Interactive Tree of Life (iTOL; https://itol.embl.de). Visualization in iTOL allowed for branch color adjustment, labeling of strain names, and annotation based on isolate metadata for clearer interpretation of genomic relationships.

Gene identification and functional characterization. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Putative biofilm-associated and antimicrobial resistance genes were identified from the annotated coding sequences generated by PGAP. The biological functions of the identified genes were subsequently evaluated through a comprehensive literature review and comparison with previously published studies describing their roles in adhesion, biofilm formation, virulence, and antimicrobial resistance in *E. faecium*.

Prediction of 3D protein model. The three-dimensional (3D) structures of the target proteins were predicted from amino acid sequences derived from the *E. faecium* genome, as obtained through whole genome sequencing. Since the corresponding protein structures were not available in the Protein Data Bank, structural predictions were performed using the AlphaFold2.ipynb tool available on ColabFold (https://github.com/sokrypton/ColabFold).

Structural validation of predicted protein models. The stereochemical quality of the AlphaFold2-predicted protein structures was evaluated using the PROCHECK server through Ramachandran plot analysis. The predicted protein structures generated by AlphaFold2 were converted into PDB format and submitted to PROCHECK for structural validation. The distribution of amino acid residues within the most favored, additional allowed, generously allowed, and disallowed regions of the Ramachandran plot was analyzed to assess model quality.

RESULTS

Biofilm formation assay. Prior to genomic analysis, the biofilm-forming ability of isolate MFKCC-BF.UR33 was evaluated using the MTPA. The isolate exhibited an optical density (OD₆₀₀) value of 0.9170, whereas the optical density cut-off (ODc) was 0.1005. Based on the established MTPA classification criteria (OD > 4 × ODc) (19), the isolate was classified as a strong biofilm producer. This phenotype served as the basis for selecting the isolate for subsequent whole-genome sequencing and structural analyses.

Genome identification. Whole-genome sequencing (WGS) identified isolate MFKCC-BF.UR33 as *Enterococcus faecium* with 99.61% sequence identity. The assembled genome had a total length of 3,322,669 bp, a G+C content of 37.35%, and no detectable plasmids. Genome annotation identified 2,321 predicted coding sequences (CDSs), 47 tRNA genes, and 13 rRNA genes (Table 1 and Fig. 1). Sequencing generated 96,277 reads comprising 748,601,758 bp, all of which mapped to the assembled genome, resulting in an estimated mean genome coverage of approximately 227×. The final assembly consisted of six contigs with a contig N50 value of 2,844,378 bp. Genome completeness assessment using BUSCO revealed 98.4% complete BUSCOs, including 97.6% single-copy and 0.8% duplicated genes, with only 1.6% fragmented genes and no missing BUSCO markers. These metrics indicate a highly complete and high-quality genome assembly suitable for downstream genomic analyses. The genome sequence has been deposited in the NCBI database under accession number JBGKAM000000000.

The genomic organization of MFKCC-BF.UR33 is shown in Fig. 1.

The circular genome map depicts the distribution

of annotated coding sequences across the assembled genome and highlights the locations of selected genes identified through genome annotation. Biofilm-associated and antimicrobial resistance-related genes were distributed across distinct chromosomal regions, supporting their chromosomal localization within the MFKCC-BF.UR33 genome. No plasmid-derived sequences were detected in the assembly.

Table 1. Sequencing and assembly statistics of *E. faecium* MFKCC-BF.UR33

Parameter	Value
Total reads	96,277
Mapped reads	96,277 (100%)
Total sequenced bases	748,601,758 bp
Total mapped bases	755,180,649 bp
Mean genome coverage	227×
Genome size	3,322,669 bp
GC content	37.35%
Contig N50	2,844,378 bp
Scaffold N50	2,844,378 bp
Duplication rate	13.07%
Complete BUSCOs	98.4%
Single-copy BUSCOs	97.6%
Duplicated BUSCOs	0.8%
Fragmented BUSCOs	1.6%
Missing BUSCOs	0.0%

Phylogenetic tree. The phylogenetic relationship of MFKCC-BF.UR33 to other Enterococcus species is shown in Fig. 2.

Phylogenetic analysis confirmed the taxonomic placement of isolate MFKCC-BF.UR33 within the genus Enterococcus. The isolate clustered most closely with *E. faecium* strain NBRC 100486 and was clearly separated from the strains belonging to other Enterococcus species, including *E. faecalis*, *E. durans*, *E. gallinarum*, *E. hirae*, and *E. villorum*. The observed clustering pattern supports the species identification obtained through whole-genome sequencing and indicates a close evolutionary relationship between MFKCC-BF.UR33 and other members of the *E. faecium* lineage.

Functional gene profiling. The identified biofilm-associated genes included *sgrA*, *acm*, *fss3*, *scm*, and *efbA*, all of which encode adhesins or matrix-binding proteins involved in bacterial attachment and biofilm development. These genes were dis-

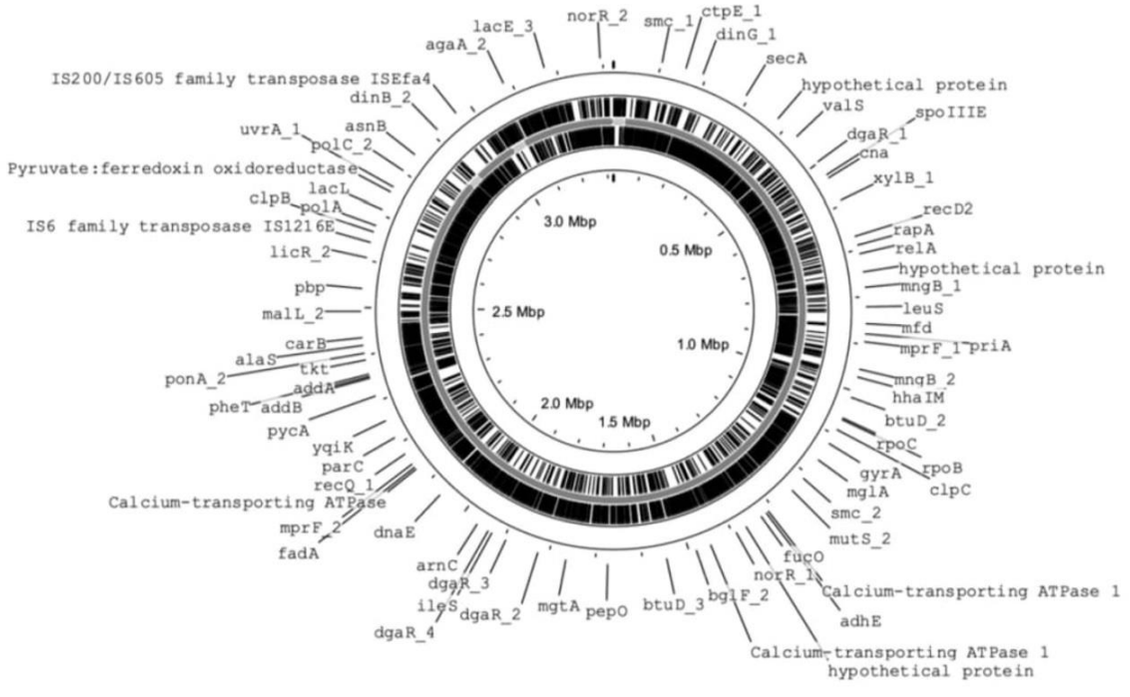


Fig. 1. Circular genome map of *E. faecium* strain MFKCC-BF.UR33 generated from the assembled genome sequence. The concentric rings represent the distribution of annotated coding sequences on the forward and reverse strands, while selected genes are labeled according to their genomic positions.

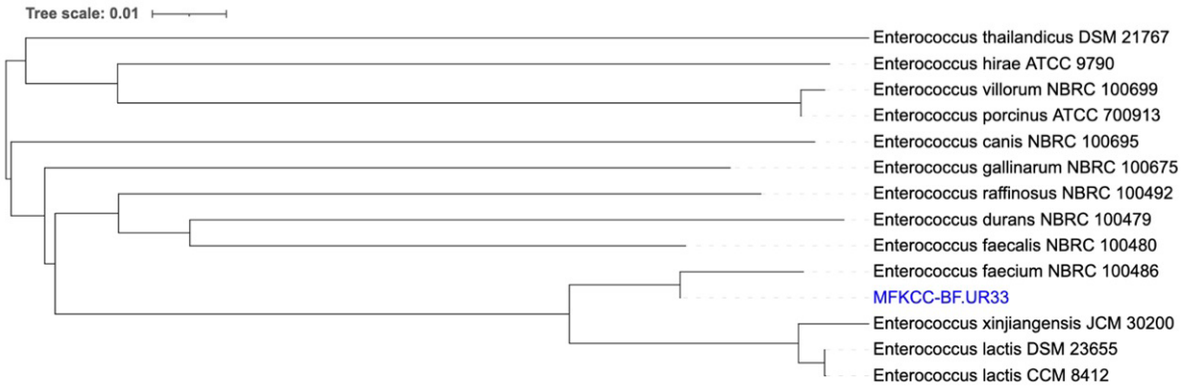


Fig. 2. Phylogenetic tree of *E. faecium* isolate MFKCC-BF.UR33 in relation to other members of the genus *Enterococcus*.

tributed across distinct chromosomal regions. The biofilm-associated genes identified in MFKCC-BF.UR33 are summarized in Table 2.

These genes encode microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and other adhesion-related proteins that facilitate bacterial attachment to host tissues and abiotic surfaces. Several antimicrobial resistance-associated genes were identified, including tetracycline resistance genes (*tet(M)* and *tet(L)*), the multidrug efflux transporter gene (*efmA*), and components of the *liaFSR* cell envelope stress response system (Table 3). The presence of these genes suggests the genetic capacity of MFKCC-BF.UR33 to support biofilm formation and antimicrobial stress adaptation.

The AlphaFold2-predicted structures of the five selected biofilm-associated proteins are presented in Fig. 3.

Structural validation of alpha fold2-predicted models. The stereochemical quality of the AlphaFold2-predicted protein models was assessed using Ramachandran plots, as shown in Fig. 4.

Fss3 and EfbA (Figs. 4C and E) show dense clustering of residues within the red (most favoured) and brown (additional allowed) regions, confirming their high stereochemical quality. In contrast, SgrA and Scm (Figs. 4A and D) display a notable concentration of residues forming a dense horizontal band in the upper region of the plot (Psi ~120-135°), outside the favoured zones, which is consistent with their

Table 2. Biofilm-associated genes identified in the genome of *E. faecium* strain MFKCC-BF.UR33

Gene	Encoding	Location
sgrA	anchored fibrinogen/nidogen-binding adhesin	2563588..2564562
acm	Collagen-binding MSCRAMM adhesin	504863..507028
fss3	Fibrinogen-binding MSCRAMM adhesin	930431..933658
scm	Collagen-binding MSCRAMM adhesin	1039752..1041734
efbA	fibronectin-binding protein	2412107..2413813

Table 3. Antimicrobial resistance-associated genes identified in the genome of *E. faecium* strain MFKCC-BF.UR33

Gene	Encoding	Location
antA/AntB	antirepressor family protein	25032..25781
Tet (M)	tetracycline resistance ribosomal protection protein Tet(M)	1342951..1344870
Tet (L)	tetracycline efflux MFS transporter	1344968..1346341
efmA	multidrug efflux MFS transporter	1646808..1648094
liaF	cell wall-active antibiotics response	1961414..1962145
liaS	two-component system sensor histidine kinase	1962142..1963209
liaX	daptomycin-sensing surface protein	10167..11735

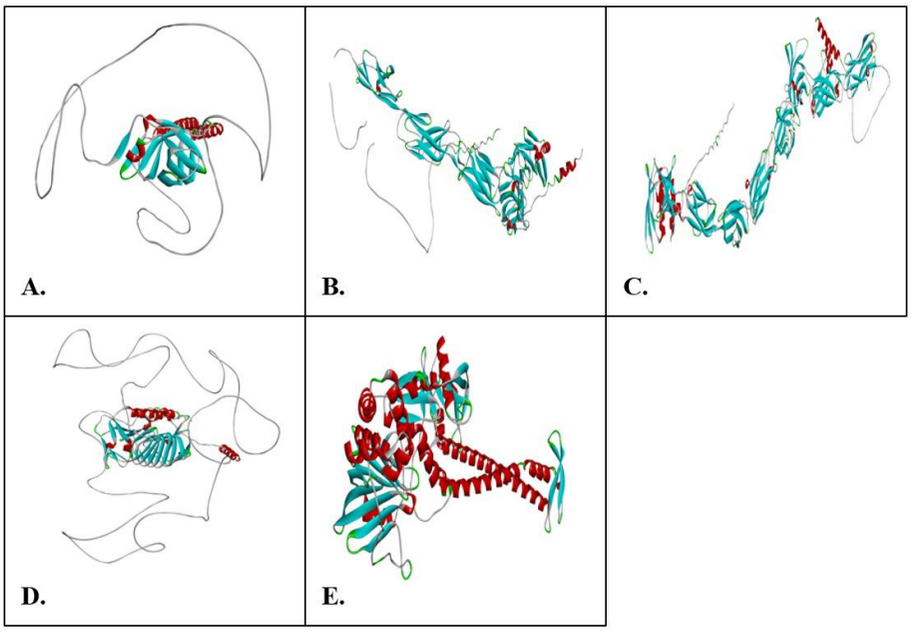


Fig. 3. Predicted three-dimensional (3D) structures of selected biofilm-associated proteins in *E. faecium* MFKCC-BF.UR33. The protein models were generated using AlphaFold2. Panels show the predicted structures of (A) SgrA, (B) Acm, (C) Fss3, (D) Scm, and (E) EfbA.

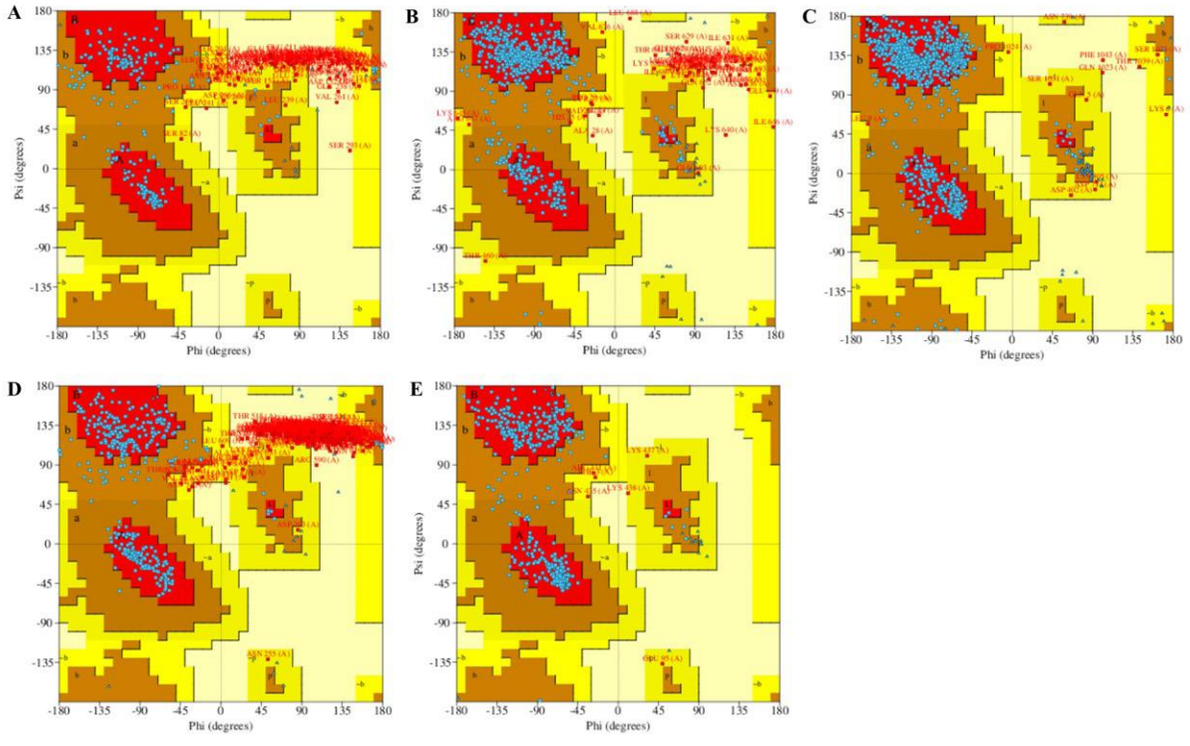


Fig. 4. Ramachandran plots of AlphaFold2-predicted protein models generated using the PROCHECK server. Panels show the stereochemical quality of: (A) SgrA, (B) Acm, (C) Fss3, (D) Scm, and (E) EfbA. Red regions indicate most favored areas; brown regions indicate additional allowed areas; light yellow regions indicate generously allowed areas; and white regions indicate disallowed areas. Internal codes in parentheses refer to PDB file identifiers generated during AlphaFold2 structural prediction.

high proportions of disallowed residues (17.00% and 34.00%, respectively).

The stereochemical quality metrics for all five predicted protein models are summarized in Table 4.

The predicted 3D structures of five biofilm-associated proteins SgrA, Acm, Fss3, Scm, and EfbA of *E. faecium* MFKCC-BF.UR33 were successfully modeled using AlphaFold2. The structural validation of AlphaFold2-predicted models using Ramachandran plot analysis demonstrated high stereochemical quality for Fss3 and EfbA, with 90.20% and 90.90% of residues, respectively, falling within the most favored regions and minimal disallowed regions. Models with >90% residues in the most favored regions are generally considered high-quality structures. Conversely, Scm and SgrA showed lower percentages in the most favored regions (46.90% and 51.00%) and relatively higher disallowed regions, indicating potential structural irregularities.

DISCUSSION

The ability of MFKCC-BF.UR33 to form strong biofilms, as demonstrated by the MTPA, supports its selection for genomic characterization. Biofilm formation is a critical virulence trait in *E. faecium*, particularly in catheter-associated urinary tract infections, where bacterial attachment and persistence on medical devices contribute to chronic infection and treatment failure. Therefore, WGS was performed to investigate the genetic determinants that may underlie the observed biofilm-forming phenotype.

WGS analysis confirmed the identity of the clinical isolate as *E. faecium* with a sequence similarity of 99.61%, consistent with species-level classification. The high sequencing depth and assembly completeness obtained in this study support the reliability of the genomic analyses performed. The assembly achieved an estimated coverage of approximately 227×, an N50 value of 2.84 Mb, complete read mapping (100%), and 98.4% completeness according to BUSCO analysis. These metrics indicate that the majority of the genomic content was successfully recovered and annotated, thereby providing confidence in the identification of biofilm-associated genes and antimicrobial resistance determinants. The high-quality assembly also minimizes the likelihood that important genomic features were missed due to incomplete genome reconstruction.

The genomic profile of *E. faecium* MFKCC-BF.UR33 provides important insights into its genetic architecture and biological functions. The assembly represented the chromosomal genome of *E. faecium* with a total length of 3,322,669 bp, a GC content of 37.35%, and no plasmids were detected. Consequently, all identified biofilm-associated genes (*sgrA*, *acm*, *fss3*, *scm*, and *efbA*) and antimicrobial resistance determinants were chromosomally encoded. Genome annotation identified 2,321 coding sequences, 47 tRNA genes, and 13 rRNA genes, which are essential for protein synthesis, cellular metabolism, and bacterial growth (20). These genomic characteristics are consistent with those reported for *E. faecium* strains and support the taxonomic assignment obtained through whole-genome sequencing (20, 21).

No plasmids were identified in the assembled genome, indicating that the detected biofilm-associated and antimicrobial resistance determinants were chromosomally encoded. This finding suggests that the key genetic traits associated with biofilm formation and antimicrobial adaptation in MFKCC-BF.UR33 are integrated within the chromosome rather than carried by extrachromosomal elements. However, additional comparative genomic analyses are required to determine the contribution of mobile ge-

Table 4. Stereochemical quality assessment of AlphaFold2-predicted biofilm-associated proteins based on ramachandran plot analysis

Protein	Ramachandran Plot Results			
	Most favoured regions (%)	Additional allowed regions (%)	Generously allowed regions (%)	Disallowed regions (%)
SgrA	51.00	17.70	14.30	17.00
Acm	77.90	13.20	4.50	4.40
Fss3	90.20	8.50	1.00	0.20
Scm	46.90	8.40	10.60	34.00
EfbA	90.90	8.00	1.20	0.0

netic elements to gene dissemination among related *E. faecium* strains. Consistent with previous reports highlighting the value of whole-genome sequencing for investigating the evolution, virulence, and antimicrobial resistance of *E. faecium* (21), the high-quality genome assembly generated in this study provides a useful resource for understanding the genomic basis of clinically relevant traits in this isolate.

The phylogenetic analysis further supported the genomic identification of MFKCC-BF.UR33 as *E. faecium*. The isolate clustered closely with reference *E. faecium* strains and was clearly distinguished from other species within the genus *Enterococcus*. This finding is consistent with previous genomic studies demonstrating that whole-genome phylogenetic analysis provides higher taxonomic resolution than conventional phenotypic methods for differentiating closely related enterococcal species. The close relationship observed between MFKCC-BF.UR33 and reference *E. faecium* strains also suggests that the isolate shares a conserved genomic background characteristic of this clinically important species.

The genomic characteristics of MFKCC-BF.UR33 were generally comparable to those reported for previously sequenced *E. faecium* strains. The genome size (3.32 Mb) and G+C content (37.35%) were consistent with values reported for both clinical and non-clinical *E. faecium* isolates (22). Likewise, the identification of multiple adhesin-associated genes, including *sgrA*, *acm*, *scm*, *fss3*, and *efbA*, is in agreement with previous studies describing the presence of adhesion- and biofilm-related determinants in pathogenic *E. faecium* strains (23, 24). Together with its phylogenetic clustering among reference *E. faecium* strains, these findings indicate that MFKCC-BF.UR33 shares core genomic features characteristic of the species. However, the combined presence of multiple biofilm-associated adhesins and antimicrobial resistance determinants provides additional insight into the genetic basis of persistence and adaptation in a biofilm-forming CAUTI isolate.

Our study identified several biofilm-associated genes in *E. faecium*, including *sgrA*, *acm*, *fss3*, *scm*, *liaX*, and *efbA*. Among these, *sgrA* encodes a surface adhesin that binds to nidogen-1 and nidogen-2, components of the basal lamina, and is known to play a crucial role in the initial attachment and subsequent development of biofilms, particularly on indwelling medical devices such as urinary catheters and prosthetic heart valves. These genes encode various resistance mechanisms such as ribosomal protection, efflux transporters, and stress response regulators. Their genomic presence supports the strain's capacity to withstand multiple antibiotic classes and highlights the genetic basis for its observed resistance phenotype, reinforcing the clinical concern surrounding biofilm-forming multidrug-resistant *E. faecium*.

SgrA has been implicated in adhesion and biofilm formation in hospital-associated *E. faecium* (23). In the present study, the identification of *sgrA* together with *acm*, *scm*, *fss3*, and *efbA* suggests that MFKCC-BF.UR33 possesses multiple adhesion-related determinants that may contribute to its previously observed biofilm-forming phenotype. Considering that the isolate originated from a catheter-associated urinary tract infection, these genes may facilitate bacterial attachment to catheter surfaces and promote persistence within the urinary tract environment.

The detection of *acm*, *scm*, and *efbA* further supports the biofilm-forming potential of the isolate, as these genes encode MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) that mediate adhesion to host extracellular matrix components. The presence of *liaX*, a gene involved in cell envelope stress response, suggests a possible regulatory link between environmental stress and biofilm production. Previous studies have shown that the expression of biofilm-related genes, such as *icaA*, significantly contributes to the biofilm-forming ability of *Staphylococcus epidermidis*, especially in implant-associated infections (25). This highlights the critical role of adhesin-encoding genes in early colonization and persistent infection, which may also be relevant in *E. faecium* pathogenesis. These findings collectively highlight the multifactorial genetic basis of biofilm formation in *E. faecium* and support the hypothesis that adhesin-related genes contribute to its persistence in hospital environments.

Another set of important genes implicated in *E. faecium* biofilm formation includes *acm*, *scm*, and *fss3*, all of which encode MSCRAMMs. These surface adhesins, along with *sgrA*, have been shown to be expressed at the mRNA level during all phases of bacterial growth (23). Their expression facilitates adhesion to host extracellular matrix proteins, contributing to the establishment and persistence of biofilms, which play a key role in hospital-acquired *E. faecium* infections. The *fss3* gene in *E. faecium* encodes a surface protein that plays a significant role

in the initial attachment of bacterial cells to abiotic surfaces, facilitating biofilm development and maturation. Genomic analyses indicate that *fss3* is often located adjacent to genes involved in extracellular polysaccharide biosynthesis. This spatial association implies a functional integration of *fss3* in the synthesis and stabilization of the biofilm matrix, suggesting its potential interaction with other extracellular matrix components to enhance biofilm structural integrity and cohesion (26, 27). The coexistence of *acm*, *scm*, and *fss3* in MFKCC-BF.UR33 indicates the presence of complementary adhesion mechanisms that may enhance colonization efficiency and biofilm stability. The simultaneous detection of these genes may provide a selective advantage for persistence on abiotic surfaces commonly encountered in healthcare settings.

Another gene of interest in biofilm formation is *efbA*, which encodes a secreted protein known as *E. faecium* biofilm-associated protein A (*efbA*). This protein facilitates the production and organization of extracellular matrix components, highlighting the crucial role that secreted factors play alongside surface-anchored adhesins in establishing the structural complexity of mature biofilms (28). The presence of *efbA* supports the hypothesis that secreted factors, in addition to surface-anchored adhesins, are essential for establishing the structural complexity of mature biofilms. The *efbA* gene has been proposed as a potential target for the development of novel therapeutic strategies aimed at disrupting biofilm formation in *Enterococcus* species. Notably, EfbA has been implicated in bacterial adhesion and biofilm formation, supporting its potential role in biofilm-associated infections (29). This inducible expression mechanism underscores the adaptive capability of *E. faecium* in response to antimicrobial pressure and may contribute to its persistence in clinical environments. The identification of *efbA* alongside several surface-associated adhesins suggests that both secreted and surface-anchored proteins may contribute to biofilm development in this isolate. This combination of biofilm-associated determinants may partially explain the ability of *E. faecium* to establish persistent device-associated infections.

In-depth genomic analyses, such as those reported by (30), have provided valuable insights into the genetic diversity and antibiotic resistance mechanisms of *E. faecium*. Notably, the presence of specific resistance genes has been associated with enhanced biofilm formation, which further increases the organism's intrinsic resistance to antimicrobial agents (31). In our study, several resistance-associated genes were identified, including *ant*, *tet*, *efmA*, and *lia*. These genes are known to encode aminoglycoside-modifying enzymes (*ant*), tetracycline resistance proteins (*tet*), multidrug efflux pumps (*efmA*), and cell envelope stress response regulators (*lia*), respectively.

Tetracycline resistance in *E. faecium* is primarily mediated by the presence of *tetM* and *tetL* genes, which encode ribosomal protection proteins and efflux pumps, respectively. Genotypic studies have shown that *tetM* and *tetL* are present in 95.5% and 57.7% of isolates, respectively (32). These genes are often located on mobile genetic elements, contributing to their widespread dissemination. Interestingly, a recent study identified a novel small RNA, *ern0030*, encoded within the 5′ untranslated region of *tetM*, suggesting a potential regulatory role in resistance gene expression (33).

Components of the LiaFSR/LiaX envelope stress response pathway identified in this study included liaF, liaS, and liaX. Recent evidence indicates that this system contributes to resistance against anti-*Enterococcus* agents such as daptomycin and bacitracin (34, 35). Collectively, the presence of *tet(M), tet(L), efmA*, and the *liaFSR* system indicates that MFKCC-BF.UR33 harbors multiple genetic determinants associated with antimicrobial adaptation. Although these genes alone do not necessarily predict phenotypic resistance, their coexistence may enhance bacterial survival under antimicrobial pressure and contribute to the persistence of biofilm-associated infections in clinical settings.

The application of computational tools such as AlphaFold2 provides valuable insights into the structural characteristics of proteins involved in *E. faecium* biofilm formation (36, 37). The predicted structures may serve as a preliminary computational framework for identifying putative functional domains, however, experimental validation through techniques such as X-ray crystallography, cryo-EM, or molecular docking simulations is required before these proteins can be formally proposed as therapeutic targets.

Structural validation of the AlphaFold2-predicted models was performed using Ramachandran plot analysis generated by the PROCHECK server. Based on Ramachandran plot assessment using

PROCHECK (38), models with a high proportion of residues in favored regions and minimal residues in disallowed regions indicate better stereochemical quality. Among the analyzed proteins, Fss3 and EfbA satisfied this criterion, with 90.20% and 90.90% of residues located in the most favored regions and minimal residues in disallowed regions (0.20% and 0.0%, respectively), indicating high-confidence structural models. Acm demonstrated moderate stereochemical quality, with 77.90% of residues in the most favoured regions and 4.40% in disallowed regions. In contrast, SgrA and Scm exhibited substantially lower percentages of residues in the most favored regions (51.00% and 46.90%, respectively) and higher proportions of disallowed residues (17.00% and 34.00%, respectively), suggesting lower model reliability. Therefore, the structural interpretations of SgrA and Scm should be treated with caution and may benefit from additional computational refinement or experimental validation.

The high stereochemical quality observed for the Fss3 and EfbA models provides greater confidence in their predicted structural conformations and supports their use in future structure function investigations. Because both proteins have been implicated in bacterial adhesion and biofilm development, reliable structural models may facilitate the identification of conserved domains and functional regions that contribute to these biological processes. Recent advances in deep-learning approaches, particularly AlphaFold2, have substantially improved the accuracy of protein structure prediction and expanded opportunities for studying proteins for which experimental structures are unavailable (36, 39).

The successful prediction of Fss3 and EfbA structures demonstrates the utility of computational structural biology for characterizing biofilm-associated proteins in *E. faecium*. Although these models provide valuable preliminary insights into protein architecture, their biological relevance and potential applications require further validation through complementary computational and experimental approaches. Future studies incorporating molecular docking, mutagenesis, and structural determination techniques will be important for confirming the functional roles of these proteins and exploring their potential relevance in biofilm-associated infections (37, 40).

A limitation of this study is the inclusion of a single clinical *E. faecium* isolate, which may not fully capture the genomic diversity of biofilm-forming strains circulating in healthcare settings. Consequently, the identified biofilm-associated genes and resistance determinants should be interpreted within the context of this isolate. Future studies involving larger collections of clinical isolates from diverse sources are needed to validate these findings and improve our understanding of the genomic basis of biofilm formation and antimicrobial resistance in *E. faecium*.

CONCLUSION

This study provides genomic and structural insights into the biofilm-forming *Enterococcus faecium* strain MFKCC-BF.UR33 isolated from a catheter-associated urinary tract infection. Whole-genome sequencing confirmed its taxonomic identity and revealed a plasmid-free chromosome harboring multiple biofilm-associated genes (*sgrA*, *acm*, *fss3*, *scm*, and *efbA*) as well as antimicrobial resistance determinants. Structural modeling using AlphaFold2 demonstrated high-confidence predictions for Fss3 and EfbA, providing preliminary structural information for future functional and structure-based investigations. Collectively, these findings improve our understanding of the genetic and structural factors associated with biofilm formation and antimicrobial resistance in *E. faecium*. However, as this study was limited to a single clinical isolate, further investigations involving larger collections of clinical strains and experimental validation are required to confirm and extend these findings.

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