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Development of siRNA-Based Therapeutic Strategy Targeting Virulence Factor *lpfA* of Zoonotic *Escherichia coli* O157:H7

Rian Ka Praja[1]*, Oktaviani Naulita Turnip[1], Hanasia[1], Reny Rosalina[2]
[1]Department of Microbiology, Faculty of Medicine; [2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Palangka Raya. Kampus UPR Tunjung Nyaho Jalan Hendrik Timang, Palangka Raya, Kalimantan Tengah, Indonesia.

Corresponding author*
riankapraja@med.upr.ac.id

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Abstract

This study aimed to identify potential small interfering RNA (siRNA) molecules capable of silencing the lpfA virulence gene of O157:H7, a zoonotic pathogen responsible for severe gastrointestinal disease. The nucleotide sequence of lpfA from *E. coli* O157:H7 strain Sakai was analyzed, and candidate siRNAs were designed using multiple bioinformatics tools, including siPRED, siRNA Scales, MaxExpect, and DuplexFold. Top ten selected siRNA candidates demonstrated high predicted inhibitory activity, with silencing efficiencies ranging from 93.73% to 94.66%. Secondary structure predictions indicated stable folding without inhibitory intramolecular structures, while binding energy analysis showed strong siRNA–mRNA duplex stability, with the best candidate exhibiting −26.5 kcal/mol. These findings suggest that the identified siRNAs possess strong theoretical potential to suppress lpfA expression and may serve as candidates for future experimental validation. Overall, this study provides a foundational step toward developing siRNA-based therapeutic strategies targeting key virulence factors of *E. coli* O157:H7.

Keywords: lpfA gene; *Escherichia coli* O157:H; siRNA; virulence factor.

INTRODUCTION

Zoonotic diseases, those transmitted from animals to humans, constitute an increasingly recognized global public health concern. One major zoonotic pathogen with substantial impact on human health is *Escherichia coli* (*E. coli*) O157:H7 (Puligundla & Lim, 2022). This strain, classified within the Enterohemorrhagic *E. coli* (EHEC) group, is capable of causing bloody diarrhea, hemolytic uremic syndrome (HUS), and acute kidney failure, particularly among children and the elderly (Gugsa et al., 2022). In developing countries such as Indonesia, the detection of *E. coli* O157:H7 is often suboptimal due to limited diagnostic infrastructure and inadequate epidemiological surveillance. The bacterium may be isolated from various sources, including undercooked meat, raw milk, and contaminated water (Lange et al., 2022).

Numerous studies have documented the presence of *E. coli* O157:H7 in Indonesia. Research conducted in Bali in 2015 reported that among 60 fecal samples obtained from Bali cattle in Mengwi District, two samples (3.3%) tested positive for *E. coli* O157:H7, while five samples (8.3%) from another set collected in South Kuta were also positive (Praja et al., 2015). Another study identified *E. coli* O157:H7 in 2 of 30 chicken meat samples (6.7%) collected from traditional markets in Pangkal Pinang, with MPN values of 1100 and >1100 MPN/g. Significant risk factors ($P < 0.005$) associated with contamination included vendors older than 45 years, stall proximity of less than 5 meters to main roads, and the use of unclean water for handwashing. The detection of *E. coli* O157:H7 in these samples underscores its potential threat to consumer health (Nurhakim et al., 2022). Additional investigations using culture and PCR techniques have identified four positive *E. coli* O157:H7 samples from eight fecal samples of diarrheal patients (Rizky et al., 2021).

The pathogenicity of *E. coli* O157:H7 is strongly associated with the expression of several virulence determinants that enable bacterial adherence, colonization, tissue damage, and systemic toxin-mediated injury. Key virulence genes contributing to disease severity include stx1, stx2, eae, and lpfA (Kalalah et al., 2024; Robinson et al., 2006). The lpfA gene, encoding long polar fimbriae, contributes to the initial adherence phase and promotes early colonization before intimate attachment occurs. These fimbriae aid bacterial persistence in the gut environment and support biofilm formation, thereby enhancing infection persistence (Torres et al., 2009; Zhou et al., 2021).

In recent years, RNA interference-based therapies, particularly small interfering RNA (siRNA), have emerged as promising molecular strategies for precisely

silencing specific gene targets (Hu et al., 2020). siRNAs are 21–23 nucleotide double-stranded RNA molecules that mediate post-transcriptional gene silencing (Ali Zaidi et al., 2023). By designing siRNAs that specifically target virulence genes, it is theoretically possible to suppress their expression and consequently impair the pathogen's ability to infect or damage its host. The application of siRNA has been extensively investigated in fields such as cancer therapy (Sousa & Videira, 2025) and antiviral research (Chokwassanasakulkit et al., 2024; Levanova & Poranen, 2018), yet its use against bacterial pathogens, particularly zoonotic bacteria, remains limited.

Research exploring siRNA-mediated suppression of *E. coli* O157:H7 virulence factors is still scarce, despite the potential of this approach to offer highly specific therapeutics with minimal toxicity and without inducing selective pressure that drives antibiotic resistance (Motamedi et al., 2024). Moreover, siRNA-based strategies could be developed as prophylactic tools for high-risk populations such as livestock handlers, slaughterhouse workers, and individuals frequently exposed to farm environments (Crump et al., 2002; Stacey et al., 2007). This approach aligns with environmentally sustainable and biotechnology-driven zoonosis control strategies.

By designing siRNAs that selectively target major virulence genes of *E. coli* O157:H7, this research aims to introduce a novel and precise non-conventional antibacterial strategy with minimal risk of resistance development. Furthermore, the findings are expected to serve as a foundation for developing siRNA-based interventions against other zoonotic pathogens, thereby contributing to the achievement of sustainable development goals (SDGs), particularly in public health, zoonotic disease mitigation, and biomedical innovation.

MATERIALS AND METHODS

Exploration of siRNA Targeting lpfA Gene in *E. coli* O157:H7

The design of siRNAs targeting the lpfA gene of *E. coli* O157:H7 was performed using the siPRED platform, which provides functional and target-specific siRNA constructs based on updated algorithms that substantially minimize off-target silencing. Gene sequences were retrieved by downloading FASTA-formatted data from the National Center for Biotechnology Information (NCBI) database, which were subsequently used as templates for designing anti-lpfA siRNAs.

siRNA Scales Analysis

The siRNA Scales tool was employed to predict the percentage of residual mRNA remaining within cells following siRNA-mediated cleavage. The designed siRNA sequences were input into the platform together with the corresponding target RNA nucleotide sequences. The analysis output provided an estimate of the proportion of mRNA persisting after siRNA-induced degradation.

Secondary Structure Analysis of siRNA

Secondary structure prediction of the siRNAs was conducted using the MaxExpect algorithm, which generates a set of highly probable structural conformations for a given RNA or DNA sequence. Each predicted structure contains base pairs with the highest estimated accuracy. MaxExpect, accessible at https://rna.urmc.rochester.edu/RNAstructureWeb/Servers/MaxExpect/MaxExpect.html was utilized by inputting the 21-nt guide RNA oligonucleotide sequences (5′→3′) derived from the siRNA design analysis. The output included the predicted RNA secondary structure along with the corresponding free energy values.

Binding Energy Analysis of siRNA and Target

The binding affinity between siRNA and its target sequence was assessed using DuplexFold, a tool that predicts the lowest free-energy hybrid conformation formed between two RNA or DNA sequences while allowing favorable intramolecular pairing. Both the 21-nt guide RNA (5′→3′) and the 21-nt passenger RNA strands (5′→3′) were submitted for analysis. The resulting output included the predicted free binding energy and the corresponding secondary structure model of the siRNA–target duplex.

Prediction of siRNA Efficacy

Prediction of siRNA functional efficacy was carried out using siPRED (http://predictor.nchu.edu.tw/siPRED/), which estimates the expected silencing efficiency of siRNA constructs. The 21-nt guide RNA sequences (5′→3′), obtained from the siRNA design analysis, were used as input to evaluate the predicted percentage of siRNA activity.

RESULTS AND DISCUSSION

Nucleotide Sequences of the lpfA Gene of *E. coli* O157:H7

The lpfA gene represent the principal virulence determinants that play critical roles in the pathogenicity of *Escherichia coli* O157:H7. The lpfA gene encodes long polar fimbriae, a fimbrial structure essential for the initial adherence of bacteria to the intestinal mucosa and for facilitating effective colonization. At the molecular level, stx1 and stx2 consist of an A and B subunit, with the present study focusing specifically on the stx1A and stx2A regions, as these subunits constitute the catalytically active components of the toxins.

The nucleotide sequences for the lpfA genes were retrieved from the NCBI GenBank database under the accession number NC_002695.2, corresponding to *E. coli* O157:H7 strain Sakai. The sequence was retrieved in

FASTA format and examined to ensure correspondence with the *E. coli* O157:H7 strain utilized in this study. Preliminary sequence analysis indicated that the lpfA genes measure approximately 603 bp.

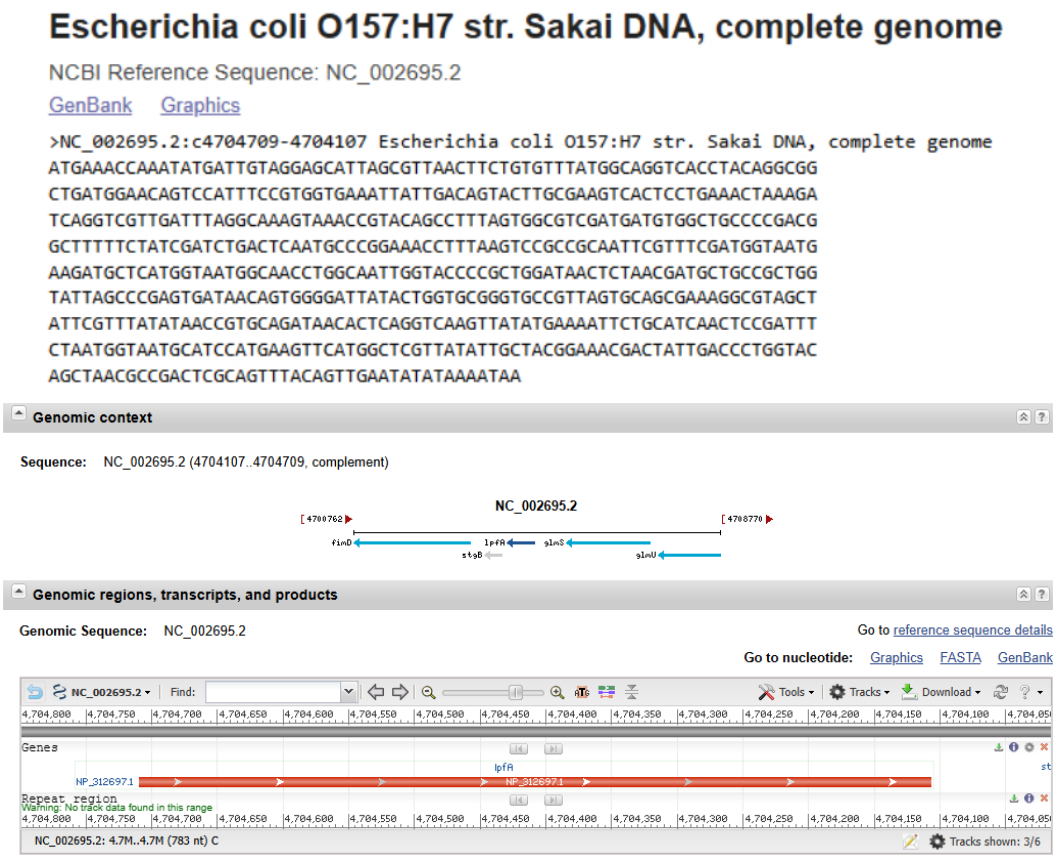

Figure 1. The gene sequence of lpfA and its position on the E. coli O157:H7 strain Sakai genome.

Identification of Potential siRNA for Silencing lpfA *E. coli* O157:H7

At this stage, the siPRED application was used to predict small interfering RNAs (siRNAs) that have the potential to silence the expression of the lpfA gene in *E. coli* O157:H7 strain Sakai. siPRED is a web-based software designed to predict the silencing potential of siRNAs against specific target mRNAs. The application considers several parameters, such as siRNA duplex stability, sequence complementarity, and the potential for inhibiting target gene expression. Each predicted siRNA candidate is assigned a score that reflects its efficiency and potential to suppress target gene expression. This score is based on factors such as the duplex stability between the siRNA and its target mRNA. The higher the score, the greater the potential of the siRNA to silence the target gene. During the identification process, an inhibition capacity >90% was used to obtain siRNA candidates with strong silencing potential. This study identified several potential siRNAs that could inhibit the expression of *E. coli* O157:H7 virulence factors, with maximum inhibition values ranging from 93.73% to 94.66%. The results of this study show the top 10 siRNAs for the target gene with the highest inhibition percentages (Table 1).

Table 1. Predicted siRNA for silencing *lpfA* gene of *E. coli* O157:H7 strain Sakai.

Rank	Antisense Strand (5'→3')	Sense Strand (5'→3')	Position	Inhibition (%)
1	UUAUUUCGCCUAAAUCAAC	GUUGAUUUAGGCAAAUGUAA	148–166	94.66
2	UAAACACAGAAGUAAUACGC	GCGUAUUACUUCU GUGUUUA	31–49	94.61
3	UUUGCCUAAUACUACAGACC	GGGUCU GUAGUAUAGGCAAA	144–162	94.61
4	UUUC CGUAGCAAUAAUACG	CGUAUUAUUGCUACGGAAA	523–541	94.59
5	UUUCCAACGUAAACUGCAC	GUGCAGUUUACGUUGGAAA	575–593	94.35
6	UAUAACGAGUAGUACUGCGC	GCGCAGUACUACUCGUUAUA	413–431	94.12
7	UAUCACGCGGCCUAAAUACC	GGGUAUUUAGGCCGCGUGAU	349–367	94.09
8	AUAUUACAGUCGUAAACGUC	GACGUUUACGACUGUAAUAU	576–594	93.98
9	UUUUCUAUUACUACGAAGCUU	AGCUUCGUAGUAAUAGAAA	452–470	93.96
10	UAAACACAGGACGUGAUCU	AGAUCACGUCUGUUGAUUUA	138–156	93.73

By using the siPRED application, several siRNA candidates with potential effectiveness in inhibiting the expression of lpfA gene in *E. coli* O157:H7 were successfully identified. These siRNAs can serve as a foundation for further experiments to test their ability to reduce the expression levels of virulence-related proteins and inhibit the processes of adhesion and invasion into host cells.

Secondary Structure of siRNA

At this stage, the secondary structure analysis of the predicted siRNA candidates was performed using the MaxExpect application. This application is used to model and verify the secondary structure of the generated siRNAs, with the aim of ensuring that the selected siRNAs do not form secondary structures that could reduce their effectiveness in inhibiting the expression of target genes. The representation result of the MaxExpect analysis for the four siRNAs are shown in Figure 2.

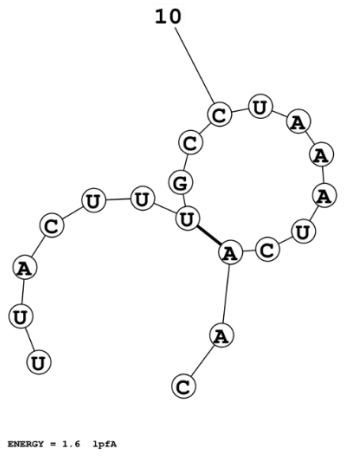

Figure 2. Representation of the secondary structure analysis results of the most inhibitory siRNA1 using MaxExpect, showing siRNA against *E. coli* O157:H7 strain Sakai lpfA with 1.6 kcal/mol.

The MaxExpect application maps the potential for secondary structure formation of the siRNA. These secondary structures include the possibility of double helices or loops forming within the siRNA sequence. The analysis results indicate that all siRNA candidates do not form significant secondary structures that could reduce their effectiveness in interacting with the target mRNA. The free energy value represents the energy required to form the secondary structure of the siRNA. Lower free energy values indicate that the secondary structure is more stable. Based on the secondary structure analysis performed using MaxExpect, it can be concluded that the representations of the selected siRNA candidates exhibit stable secondary structures with free energy values that do not hinder their ability to interact with the mRNA of lpfA of *E. coli* O157:H7 virulence factor genes. The value supported the potential of the selected siRNAs to effectively inhibit gene expression in *E. coli* O157:H7.

siRNA Binding Energy

At this stage, the binding energy analysis between the siRNA candidates and the target mRNA of *E. coli* O157:H7 virulence factors was performed using the DuplexFold application. This application is used to model the interaction between siRNA and target mRNA and calculate the energy required to form a duplex between the two molecules. This binding energy analysis is crucial for evaluating the stability of the siRNA-mRNA complex, which affects the effectiveness of the siRNA in inhibiting target gene expression.

The DuplexFold application calculates the duplex binding energy based on the interactions between siRNA and target mRNA. This energy is computed by considering base pair stability, double helix structure, and the potential formation of loops that could disrupt binding. The analysis results show that the three siRNA candidates can form duplexes with the target mRNA, but with different binding energy values, reflecting the strength and stability of their interactions. The results of the DuplexFold analysis are shown in Figure 3.

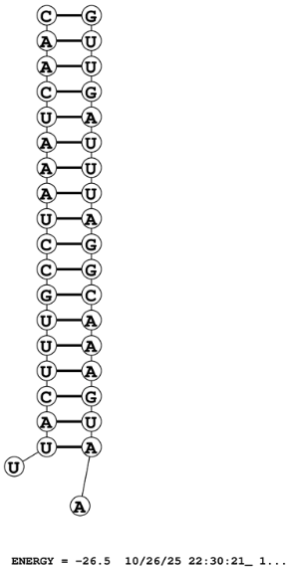

Figure 3. Representation of the DuplexFold analysis results for siRNA against *lpfA E. coli* O157:H7 strain Sakai with -26.5 kcal/mol.

Lower binding energy values indicate that the interaction between siRNA and target mRNA is more stable, meaning the siRNA will bind more easily to the target mRNA and be more effective in inhibiting gene expression. Higher binding energy values may indicate a less stable interactions, potentially reducing the effectiveness of the siRNA.

Discussion

This study aims to identify potential siRNAs capable of silencing the expression of lpfA gene in *E. coli* O157:H7, which play a crucial role in bacterial motility to host cells. Several bioinformatics methods were used to analyze the gene sequences, predict siRNAs, analyze siRNA secondary structures, and evaluate the binding energy between siRNA and the target mRNA. The identification results of the lpfA gene from *E. coli* O157:H7 strain Sakai, obtained from GenBank, show that these genes encode fimbriae, which are involved in the pathogenesis of *E. coli* O157:H7 in epithelial host cells (Zhou et al., 2021). The lpfA gene contain conserved and crucial regions for the bacteria, making them key targets in efforts to block infection. Therefore, the gene were selected as targets in this study for regulation using siRNA. The sequences of lpfA were obtained from the GenBank database (NCBI) under accession number NC_002695.2, corresponding to *E. coli* O157:H7 strain Sakai. Initial analysis of the sequences revealed that the lpfA gene are approximately 603 bp in length.

The siRNA prediction results using the siPRED application identified several potential siRNA candidates targeting the lpfA gene in *E. coli* O157:H7. However, 10 siRNAs with the best inhibitory potential for the gene were selected. Candidates such as siRNA lpfA1 (5'-UUACUUUGCCUAAAUCAAC-3') showed high potential in silencing the expression of lpfA gene, based on high efficiency scores (Pan et al., 2011). The siPRED application selects siRNA candidates with high stability in binding to the target mRNA, which is essential for ensuring successful silencing. Previous studies also showed that siRNA prediction using bioinformatics tools such as siPRED can identify effective siRNAs targeting specific genes and can reduce the cost and time needed for laboratory experiments. The results obtained from this application provide a solid foundation for further experiments to evaluate the siRNA efficiency in in vitro conditions (Nur Islam et al., 2025; Rao et al., 2025).

Secondary structure analysis of siRNA using the MaxExpect application showed that all selected siRNA candidates have stable secondary structures without forming double helices or loops that could hinder binding with the target mRNA (Lu et al., 2009). The free energy and minimal folding free energy (MFOLD) values calculated by MaxExpect for each siRNA candidate showed optimal stability. For example, siRNA1 lpfA had free energy values of 1.6 kcal/mol, indicating good stability and the potential for siRNA to function effectively in the silencing process. Previous research has also shown that stable secondary structures in siRNA enable better interaction with target mRNA, potentially enhancing silencing efficiency (Al-madhagi, 2024).

Binding energy analysis between siRNA and the target mRNA using the DuplexFold application provided insights into the stability of the siRNA-mRNA interactions for various virulence factors. The results indicated that the designed siRNAs had the highest binding energies for each gene (-26.5 kcal/mol), suggesting more stable interactions with the target mRNA. Lower binding energies indicate that the siRNA will bind more easily to the mRNA. Previous studies that used DuplexFold to analyze binding energy between siRNA and mRNA also showed that siRNAs with lower binding energies tend to be more effective in inhibiting target gene expression. These results confirm that siRNA 3 is the best candidate based on binding stability and high silencing potential (Al-madhagi, 2024).

CONCLUSIONS

This study successfully identified several potential small interfering RNA (siRNA) candidates capable of targeting and silencing the lpfA virulence gene of *Escherichia coli* O157:H7 using an integrated bioinformatics approach. Sequence retrieval and analysis confirmed the conserved structure of the lpfA gene, supporting its suitability as a therapeutic target. Predictive computational tools identified ten siRNA candidates with high inhibitory potential, demonstrating silencing efficiencies above 93%. Secondary structure modeling showed that these siRNAs possessed stable conformations favorable for effective gene targeting, while binding energy analysis indicated strong duplex formation between siRNAs and target mRNA. Collectively, these results suggest that the

predicted siRNAs have strong theoretical potential to suppress lpfA expression and may contribute to inhibiting early bacterial adhesion and colonization. The findings lay the groundwork for further in vitro and in vivo studies to validate their therapeutic applicability and advance siRNA-based strategies against zoonotic *E. coli* O157:H7.

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Competing Interests: The authors declare that there are no competing interests.

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