

Article

Enhancing Vaccine Efficacy Against Staphylococcus Aureus Infections Using Glycoside Hydrolases

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Abstract: Staphylococcus aureus is one of the most clinically significant Gram-positive bacterial pathogens, frequently colonizing the skin, nasal cavity, throat, and gastrointestinal tract of humans and animals. It is a leading cause of abscesses, purulent infections, and severe systemic diseases worldwide. Although vaccination represents a promising prophylactic strategy, the formation of bacterial biofilms poses a formidable barrier that prevents vaccine candidates from accessing target antigens on the bacterial surface. To address this challenge, this study investigates the potential of glycoside hydrolases, particularly PelA, as enzymatic agents capable of degrading biofilm matrices and thereby exposing hidden antigens to the immune system. Collectively, following with the past articles. Those findings suggest that PelA-mediated biofilm degradation may enhance antigen accessibility and improve immune recognition. This study provides a mechanistic foundation for integrating glycoside hydrolases into vaccine development strategies and offers a potential approach for overcoming biofilm-associated barriers in Staphylococcus aureus immunization.

Keywords: staphylococcus aureus; vaccine; biofilm; glycoside hydrolases; protein expression

1. Introduction

Staphylococcus aureus, belonging to the genus Staphylococcus, is a representative of Gram-positive bacteria [1, 2]. This bacterium is a type of pathogenic bacteria that often colonizes the skin, nasal cavity, throat, gastrointestinal tract, abscesses, and purulent lesions of humans and animals, and is widely present in environments such as air and wastewater. Infectious diseases caused by it remain a major global health challenge and continue to pose a significant threat to human health worldwide. Although a complex global health system has been established to respond to known and emerging infectious disease threats, many infectious diseases persist, recur, or emerge over a long period of time. Currently, vaccines for treating this bacterial infection are also an effective method. Recently, researchers designed a bivalent self-amplifying mRNA (saRNA) vaccine combination targeting the virulence antigens ClfA, Hly, and SraP of Staphylococcus aureus. A comprehensive framework combining immunoinformatic, structural bioinformatics, molecular simulation, and database-curated experimental benchmark data was employed to identify the immunodominant epitope regions of each antigen. Ultimately, two saRNA vaccine candidates, SaBVax807 and SaTVax876, were rationally designed. Molecular docking and molecular dynamics simulations were then conducted to characterize and validate the binding interactions between the two constructs and anti-S. Population coverage analysis of SaTVax876 was utilized to assess its global applicability. The vaccines in each candidate region were codon-optimized to maximize the transformation efficiency of human host cells, and a comprehensive evaluation of allergenicity, antigenicity, autoimmune risk, physicochemical properties, toxicity characteristics, and molecular interaction dynamics was conducted to rigorously assess safety, stability, and immunogenicity potential. This evaluation demonstrated that SaRNA vaccines can become one of the primary vaccines for antibacterial purposes [3]. However, the extracellular polymeric substance (EPS) matrix acts as a physical barrier,

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which prevents pathogens from being recognized by immune cells such as macrophages and neutrophils. This structural barrier reduces the immune recognition efficiency of vaccines, shields bacterial antigens, and enables pathogens to evade immune clearance [4]. Meanwhile, enhanced antibiotic resistance and immune evasion capabilities enable bacterial biofilms to persist for extended periods, which reduces effective immune recognition and limits antigen accessibility. For this reason, methods to disrupt targeted biofilms have been proposed as a promising strategy for the prevention and treatment of infectious diseases associated with *Staphylococcus aureus* infections [5]. In recent years, glycosyl hydrolases have emerged as a primary biochemical tool in enzymatic degradation reactions. Previous studies have confirmed that glycosyl hydrolases exhibit strong degradation capabilities against *Pseudomonas aeruginosa* biofilms and facilitate the diffusion of embedded bacteria, thereby enhancing bacterial sensitivity to antibiotics. In the future, this enzyme, which decomposes cell membranes producing different products, could serve as a primary therapeutic approach. This enzyme can simulate environments with varying nutrient compositions, oxygen levels, and pH values, unlocking its potential to treat biofilm-related infections. Currently, scientists have limited understanding of the composition of EPS in the presence of multiple species or whether it changes due to variations in the biofilm growth environment. Identifying the chemical composition of EPS remains a significant challenge in the future, necessitating further experiments to validate this viewpoint [5]. In this scholar paper, I will show about using Pel A a kind of GH and illustrate about the feasibility and how to enhance about future discovering.

2. Present testing

Currently, this enzyme is still under experimentation, some experiment clearly shows this kind of enzyme has an obvious function of restressing *Staphylococcus Aureus*. This study selected 33 soil samples from subtropical regions to screen for functional strains producing pectate lyase. In the initial screening stage, soil samples were prepared into uniform bacterial suspensions, diluted in a gradient manner, and plated onto a selective medium containing orange peel powder as the sole carbon source. The cultures were then incubated at 37°C for 48 hours. Based on colony morphology differences, individual colonies were picked and serially passed on for purification until stable pure strains were obtained. During the re-screening process, the purified strains were inoculated into liquid selective medium and incubated at 37°C and 220 rpm for 48 hours. After centrifugation of the fermentation broth, the supernatant was collected, and the pectate lyase activity of each strain was measured. The strains B12-7 and B15-1, which exhibited the highest enzyme activity, were finally selected as materials for subsequent experiments. In this study, the PelA enzyme activity was measured using a 235 nm ultraviolet spectrophotometer, with polygalacturonase acid as the reaction substrate. After preheating with 280 µL of substrate buffer, an appropriate amount of diluted crude enzyme solution was added and incubated at 37°C for 10 minutes. Subsequently, a phosphoric acid solution was added to terminate the enzymatic reaction, and a high-temperature inactivated enzyme system was set up as a blank control. The enzyme activity values were calculated using a correction formula, and the enzyme activity unit was defined as the amount of enzyme required to catalyze the production of 1 µmol of unsaturated polygalacturonase acid per minute. To explore the enzymatic characteristics of the pectate lyase produced by the strains, various buffer systems spanning pH 3.0 to 11.0 were prepared, and the enzyme activity was measured under constant temperature conditions at 50°C in different pH environments. Based on the optimal pH, a temperature gradient from 30°C to 70°C was set to measure the relative enzyme activity at different temperatures. The highest enzyme activity was set as 100%, thereby determining the optimal reaction temperature and pH conditions for the enzyme. The strains were preliminarily identified by combining morphological and physiological biochemical characteristics, observing colony morphology and cell microstructure, and determining the physiological characteristics of the strains through multiple indicators such as Gram

staining, V-P test, methyl red test, carbon source sugar fermentation test, catalase and amylase activity tests. Subsequently, genomic DNA was extracted from two bacterial strains, and PCR amplification was performed using universal 16S rRNA primers. After passing the agarose gel electrophoresis test, the samples were sent for sequencing. Sequence homology alignment was conducted using the NCBI database, and a phylogenetic tree was constructed using Mega11 software to complete the molecular biological identification of the strains. Based on the strain identification, the *pelA* functional gene was amplified using its genomic DNA as a template. The target fragment was recovered and purified, then ligated with the pET-28a expression vector to construct a recombinant expression plasmid, which was transformed into competent *E. coli* BL21(DE3) cells. After resistance screening and sequencing verification, the recombinant engineering strain was cultured to a suitable cell concentration, and PelA protein was heterogeneously expressed through IPTG low-temperature induction. The cells were collected and subjected to ultrasonic disruption to prepare crude enzyme solution. The recombinant protein was purified using a nickel affinity chromatography column, and the purification effect of the protein was detected by SDS-PAGE electrophoresis. Finally, industrial cane sugar syrup was used as the treatment target, and a gel removal experiment was conducted under the optimal reaction conditions of PelA enzyme. A no-enzyme blank control was set up, and the practical application effect of recombinant PelA pectate lyase in cane sugar syrup refining and gel removal was comprehensively evaluated by detecting the residual pectin content, transmittance, and viscosity changes in the syrup [6]. In medical field, this method has already been used to test its ability to break biofilms. For example, one experiment, after the researchers had cultivated the biofilm, they carefully removed the culture medium containing planktonic cells and used PBS to rinse out excess small bacteria from the pores. Subsequently, PelA was prepared as an enzyme solution and added to the pores containing the formed biofilm. The laboratory temperature was maintained at 37°C, thereby facilitating enzymatic degradation of the extracellular polymeric substances (EPS) in the biofilm matrix. During this reaction process, glycosyl hydrolases can catalyze the hydrolysis of glycosyl bonds in the extracellular polysaccharides, which are core structural components of biofilm matrices. Through enzymatic degradation, they indirectly disrupt the structural integrity of biofilms and promote biofilm dissociation. Based on this, it can be expected that glycosyl hydrolase treatment can significantly disintegrate the overall structure of biofilms, resulting in a significant decrease in polysaccharide staining signals observed under confocal microscopy. The study used biofilms without enzyme treatment as a blank control. Through comparative analysis between groups, it was confirmed that active glycosyl hydrolases can effectively reduce the structural integrity of the extracellular matrix and enhance antigen accessibility. This result provides important experimental support for research strategies aimed at optimizing and improving vaccine efficacy through biofilm degradation. Additionally, this study employed crystal violet staining to quantitatively analyze the biomass of biofilms after PelA enzyme treatment. After incubating cells with PelA enzyme for 2 hours, the residual content of biofilms constructed from Pel-dependent components was quantitatively detected and statistically analyzed to further verify the degradation effect of the enzyme on biofilms [7]. In future studies, this kind of enzymes can be tested whether it can be used in vaccine.

3. Future Study

This study demonstrates that PelA-induced biofilm degradation serves as a viable and promising strategy for boosting vaccine immune efficacy, though this innovative approach still faces certain limitations that require systematic evaluation despite its unique advantages. Existing studies have confirmed that biofilm matrices can interfere with the interaction between pathogens and host cells, blocking bacterial contact with host tissues and thereby hindering effective immune recognition. Moreover, biofilm accumulation facilitates persistent bacterial colonization and weakens host immune clearance capacity, which further verifies that biofilm structure severely restricts antigen

accessibility. Accordingly, targeted disruption of biofilm structure can expose more antigens to the host immune system and optimize immune recognition efficiency [8]. If the present experiment achieves the expected results, it will validate that enzymatic degradation of biofilms can effectively improve antigen accessibility while reducing vaccine dosage costs, providing a novel idea for the optimization and development of bacterial vaccines. Despite the continuous progress of research on *Staphylococcus aureus* vaccines and the gradual advancement of clinical trials, the development of high-efficiency protective vaccines still faces enormous challenges. The complex adaptive mechanisms of pathogens, diverse immune evasion strategies and technical transformation difficulties have become major bottlenecks in vaccine research, indicating that traditional incremental optimization methods are far from meeting clinical application requirements. In future research, the integration of multiple emerging technologies including systems biology, reverse vaccinology, artificial intelligence and structural vaccinology can construct a more standardized and data-driven vaccine development system. These modern technical means help screen conserved immunodominant antigens, optimize epitope matching and design, and develop multivalent vaccine formulations that can trigger diversified host immune responses. Combined with the biofilm disruption strategy mediated by PelA enzyme, which improves antigen exposure efficiency, these advanced vaccine development frameworks can significantly enhance host immune recognition ability and promote the development of clinically applicable high-efficiency vaccines [9]. Enzymatic biofilm degradation can effectively break the barrier of insufficient antigen exposure, achieve effective immune protection at lower antigen doses, and provide a new solution for vaccine optimization. In essence, the improvement of vaccine efficacy based on PelA-mediated biofilm remodeling solves a key pain point in current vaccine research: the immunogenicity of antigens does not completely equate to protective immune effects, and insufficient antigen accessibility is one of the core factors restricting the design and development of high-efficiency vaccines [10]. As a cutting-edge vaccine development technology, multivalent mRNA vaccines can induce coordinated humoral and cellular immune responses and have broad application prospects, but they still have inherent defects such as limited strain coverage, antigen competitive inhibition and insufficient long-term immune protection, leaving huge room for improvement in antigen presentation efficiency and immune persistence. Biofilm disruption represented by PelA enzymatic treatment can effectively compensate for the above deficiencies by improving antigen accessibility, thereby optimizing vaccine immune efficacy [11]. Nevertheless, the limitations of this study cannot be ignored. Like most pharmacological basic research, this experiment only carries out in vitro exploratory detection. Although in vitro biofilm models have the advantages of simple operation, low cost and strong scalability, which are conducive to high-throughput screening and verification of new antibacterial strategies, they have obvious simplification defects. Most in vitro biofilm culture relies on artificial non-biological substrates, and the constructed biofilm structure is quite different from the in vivo growth state under the action of host microenvironment and physiological factors, resulting in poor predictive effect of in vitro experimental results on clinical antibacterial efficacy and treatment success rate. It is undeniable that biofilms play a vital regulatory role in host infection and pathological processes, and the in vivo microenvironment greatly affects the formation and functional characteristics of biofilms [12]. In addition, enzymatic treatment is restricted by strict reaction conditions. At present, industrial enzyme research is committed to improving enzyme activity and stability to reduce production costs and improve application sustainability. However, complex and variable industrial reaction environments such as unstable pH and temperature will seriously affect enzyme activity and utilization efficiency. Improving the environmental adaptability of enzymes has always been a key research direction in enzyme engineering. With the continuous maturity of enzyme engineering, protein engineering and genetic modification technology, it is feasible to screen acid-base and temperature-stable novel enzymes, construct mutant enzymes with strong environmental adaptability through genetic

engineering technology, and optimize the balance between enzyme structural stability and catalytic activity. This series of optimization measures can effectively improve the environmental tolerance of PelA enzyme, reduce the restriction of extreme reaction conditions, and further improve its practical application value [13]. Future mRNA vaccine research will focus on optimizing antigen design, improving structural stability and developing AI-assisted intelligent vaccine manufacturing technology. On this basis, further improving antigen accessibility will become an important breakthrough direction for enhancing vaccine efficacy. The PelA-mediated biofilm remodeling strategy can break the biofilm physical barrier, increase antigen exposure level, and then activate stronger host immune responses. The combination of this biofilm optimization strategy and mature mRNA vaccine platform can maximize the utilization efficiency of antigens and significantly enhance immune protective effects [14]. In summary, PelA enzyme-based biofilm degradation technology provides an innovative auxiliary means for vaccine development. Although the technology is still in the exploratory stage and its practical application effect needs further in vitro and in vivo verification, with the continuous innovation and maturity of modern biotechnology, this novel strategy is expected to become an efficient and economical technical means to optimize vaccine efficacy and promote the development of high-efficiency antibacterial vaccines in the medical field.

3. Conclusion

In Conclusion, Glycosidase-based biofilm disruption has emerged as a novel and viable adjuvant strategy to boost the protective potency of *Staphylococcus aureus* vaccines. By enzymatically remodeling the structural integrity of the biofilm matrix, glycosidase treatment effectively eliminates the physical barrier that shields *S. aureus* from host immune surveillance, thereby improving bacterial antigen exposure and reversing pathogen-driven immune evasion. Targeting extracellular biofilm components provides a facile and robust approach to facilitate efficient immune recognition and bacterial clearance, which can be synergistically combined with emerging next-generation vaccine modalities to optimize immunoprotected outcomes. Further mechanistic investigations into the structural and functional characteristics of PelA enzyme, as well as its molecular interactions with bacterial surface components, will support the rational design and precise application of biofilm-targeted adjuvant strategies in future vaccine development. With continuous progress in interdisciplinary biotechnology and the iterative optimization of large-scale production systems, glycosidase-mediated biofilm modulation holds great translational potential to advance the development, industrial manufacturing, and clinical transformation of high-efficiency *S. aureus* vaccines.

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