Chapter 1

Introduction

1.1 Background

In early December 2019, an outbreak of a severe respiratory illness with an unknown etiology closely resembling viral pneumonia first emerged in Wuhan, China (Kim et al., 2020). The causative agent of this infection was identified as the severe acute respiratory coronavirus (SARS-CoV-2), which has spread rapidly across the globe, causing the ongoing coronavirus disease 2019 (COVID-19) pandemic (A. Sharma et al., 2020). To date, 6.5 million deaths from 633 million COVID-19 cases worldwide have been reported due to the severe outcome of this disease (WHO, 2022b), which further implies the need for preventive measures to protect against the SARS-CoV-2 infection and transmission.

Vaccination, which elicits immune responses against a disease-causing pathogen, has been strongly advocated in order to provide society with adequate immunity against future infections and outbreaks (Clem, 2011; Omer et al., 2020). Subsequently, around 70 percent of the world's population has received at least one dose of the COVID-19 vaccine, which predominantly contains the spike (S) protein as its primary target (Funk et al., 2020; Heinz & Stiasny, 2021; Mathieu et al., 2020). The S protein, composed of the S1 and S2 subunits, is regarded as the essential component of SARS-CoV-2 in facilitating its entry by binding to the host Angiotensin Converting Enzyme-2 (ACE-2) receptor (Jackson et al., 2022). However, due to its constant mutation, especially on the latest SARS-CoV-2 Omicron (B.1.1.529) variant that harbors over 30 mutations in the S protein, the vaccine effectiveness has been shown to decrease significantly (Ou et al., 2022; Willett et al., 2022). Thus, the idea to target a more conserved region of SARS-CoV-2, in addition to S, has become the main interest of this research to provide broader protection against the rising SARS-CoV-2 variants of concern (VOCs), which might possibly originate from the current Omicron subvariants under monitoring.

In the search for a new vaccine target, the nucleocapsid (N) protein has been strongly proposed since it has been proven to acquire lower mutation rates with higher stability than the S protein (Thakur et al., 2022). Despite the initial intracellular localization of the nucleocapsid (N) protein (Bai et al., 2021), latest research has shown its presence on the surface of the infected cells, making it a potential vaccine target (López-Muñoz et al., 2022). Subsequently, multiple studies have demonstrated the ability of the N protein to induce high levels of antibodies and robust T-cell responses that can potentially provide long-lasting immunity (Ahn et al., 2022; Hajnik et al., 2022; Smits et al., 2021). Therefore, N-based proteins have recently been named as the next generation of COVID-19 vaccine targets that are believed to overcome the future emergence of SARS-CoV-2 variants (Feng et al., 2022; Oronsky et al., 2022). While it is equally important to employ the S-antibodies to suppress the viral entry at the mucosal level, by targeting the Spike-1 (S1) protein subunit that is most immunogenic compared to the Spike-2 (S2) protein subunit or whole S protein (Sahin et al., 2020; Walsh et al., 2020; Y. Wang et al., 2021). Therefore, the dual Spike-1-Nucleocapsid (S1-N) subunit vaccine was proposed to provide optimal immune coverage against SARS-CoV-2.

Nevertheless, despite its broad coverage, the binding affinity of the dual S1-N subunit vaccine towards the immune cell receptor in stimulating the immune response against SARS-CoV-2 remains unknown. At the same time, this remains one of the essential parts of ensuring the vaccine's effectiveness in recognizing and neutralizing the target. Thus, this project was the first to identify and assess the immunogenic epitopes of S1 and N subunits across SARS-CoV-2 VOCs and their subvariants using a multivalent concept where only their conserved epitopes across the variants were selected (Guest, 2022). Constructing a multivalent and multi-epitope peptide vaccine against the dual S1 and N of SARS-CoV-2, to which it was hypothesized to achieve stability for effective binding to stimulate immune responses and safety for its administrations. Likewise, for the individual S1 and N peptide vaccines built from this research using the immunoinformatics approaches.

At last, in attempts to create a vaccine, it is equally essential to determine its potential delivery method. Most of the COVID-19 vaccines available on the market are administered intramuscularly (IM) to provide systemic protection (Donnelly, 2017; Thomas, 2021). However, the IM delivery could not induce mucosal immunity (MI) in the nose and lungs, which has the potential to neutralize the virus at the initial infection sites and prevent its transmission (Azzi et al., 2022; Chandrasekar et al., 2021; Sheikh-Mohamed et al., 2022). Thus, the mucosal immune response against the dual S1-N vaccine designed was assessed *in silico*, which will be cloned inside the food-grade *Lactococcus lactis (L. lactis) NZ3900* in the following study as the host to deliver the vaccine into the gastrointestinal (GI) tract (Song et al., 2017). In the hope of stimulating the production of a neutralizing agent in the gut that would eventually migrate to distant mucosal sites such as the respiratory mucosa and other systemic effector sites (Jazayeri et al., 2021). Therefore, unraveling the potential of acquiring a multivalent and multi-epitope oral vaccine capable of inducing both mucosal and systemic immunity against S1 and N is crucial in the fight against the emerging SARS-CoV-2 variants.