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Probiotics mediated control and immunomodulation of SARS-CoV-2 associated pathological conditions

P. Saravana Kumari◆, V. Viswapriya, M. Lydia Rajakumari* and S. Murugan**

Department of Microbiology, Rathnavel Subramaniam College of Arts and Science, Coimbatore-641402, Tamil Nadu, India

**Department of Microbiology, Indira Gandhi College of Arts and Science, Kathirkamam, Pondicherry-605009, India*

***Department of Biotechnology, Karunya Institute of Technology and Sciences, Coimbatore-641114, Tamil Nadu, India*

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Abstract

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a fast-evolving respiratory infection-causing virus. The simple structure of the virus enables it to spread through the air and easy lodging into the respiratory tract of the host. The virus-specific spike protein rendering antigenicity and pathogenicity in the host. The spike protein is highly evolving as an immunogenic form through mutation. Variants of the virus are much more pathogenic that induce inflammation during the early stages of infection and cytokine storm in later stages of infection, which cause cytolysis of own host cells. Drugs used are targeted towards spike protein and virus-specific components and several drugs are under trial. A drug that needs to control the host immune response and maintain homeostasis in the host is required. Anti-inflammatory drugs and steroids recommended for immunostimulatory and as immunosuppressors according to the prevailing condition of infection. Several phytomedicines evaluated for their efficiency to control the pandemic. Another natural system to control viruses is the probiotic organisms, proven for their ability to competitively inhibit entry and establishment of viral pathogens in the host. Administration of probiotics during the primary stage of viral infection improves the host immunity and prevents the increase in viral load and in later stages, regulates the host immunity when host immunoregulation disrupted by viral antigen. This helps in the immunomodulation of the host immune system on the whole. The present review evaluates the immunomodulatory effect of probiotics to control SARS-CoV-2.

1. Introduction

The coronavirus disease 2019 (COVID-19) has imposed major threat to the children, pregnant women, employees, executives, geriatric, patients with chronic complications, researchers and industries. It affected the teaching-learning process, availing necessary health-care support at times and high risk of getting infection to the mother and infant, regular income, overall productivity and improvisation, psychological effect on safety and well-being, survival of patients with lung problem, hypertension and heart diseases, efficient technology to develop drugs and vaccines (required within short period) and challenged the scientist in mass production of drugs and vaccine to meet huge mass at a short stretch. A mild to moderate respiratory illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected all sectors of human life invariably. Prime mode of transmission is through air, as respiration is indispensable for all life, while respiring aerosols or droplets containing virus lead to the complications associated with COVID-19 infection and severe infection in persons with comorbidities. Coronavirus belongs to the family Coronaviridae, subfamily Nidovirales. A comprehensive taxonomic analysis of the viruses in the coronoviridae revealed that humans are the only host for SARS-CoV-2 (Evgeny *et al*., 2020). The first outbreak of COVID-19 and related respiratory sickness was reported in Wuhan, Hubei Province, China during December 2019. The rate of infections is very high and spread across the globe as pandemic. The World Health Organization (WHO) is to keep in track of this pandemic and disseminating relevant updated information continuously. Due to its severity in infection and the high rate of infection, the WHO on January 30, 2020 declared that the SARS-CoV-2 epidemic is a public health emergency of international concern (PHEIC). After that, WHO officially named SARS-CoV-19 infection in human as coronavirus disease-2019 (COVID-19) on February 11, 2020 and the International Committee on Taxonomy of Viruses (ICTV) named the virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The WHO reported that globally 162.7 million confirmed cases of COVID-19, including 3.3 million deaths on 17 May 2021 and around 1,264,164,553 vaccine doses administered to beneficiaries till 11 May 2021. The average number of transmission of SARS-CoV-2 from one infected person to another (R or R0) varied from 1.8 to 3.6 (average of 2.5) across the globe, comparatively higher than the recent pandemics of SARS-CoV (R-value 2.0 to 3.0) and influenza (R-value 1.5) (Petersen *et al*., 2020). Although, the R number across the UK is now between 0.7 and 0.9, in Scotland's estimated R number is between 0.8 and 1.0, in Wales the number is between 0.6 and 0.9, Northern Ireland was 0.75 and 0.95, England, was between 0.8 and 1.0 and in India it is increased from 1.8 to 2.1 recently. Comparatively, the United States, India, and Brazil are the three countries with the largest cumulative number of confirmed cases in the world. Now, SARS-CoV-2 is recognized as the third

Corresponding author: Dr. P. Saravana Kumari

Associate Professor, Department of Microbiology, Rathnavel Subramaniam College of Arts and Science, Coimbatore-641402, Tamil Nadu, India

E-mail: sarankumaribs@gmail.com

Tel.: +91-9442386269

infection which is zoonotic in origin and transmitted to human of this century and affected a huge mass with 3.4% of mortality rate (Gralinski and Menachery, 2020).

2. Structure of SARS-CoV-2

Coronaviruses belong to the subfamily Coronavirinae in the family of Coronaviridae and belongs to the group beta coronavirus. The genome of SARS-CoV-2 is a single-stranded, linear, positive-sense RNA consists of 29903 nucleotides arranged in 14 open reading frames which is larger than any other RNA viruses (Ankur Das *et al*., 2021). Genetic material is coiled with phosphate and capsid protein to form nucleocapsid phosphoprotein (N) covered by an envelope which is associated with four structural proteins: membrane protein (M), spike glycoprotein (S), hemagglutinin-esterase dimer (HE) and envelope protein (E) coded by Orf 1 (266 to 21,555 nt), and sixteen non-structural proteins (nsp1"16 coded by Orf 3, 6, 7, 8, 9 and 10) (Tai *et al*., 2020) (Figure 1). Functions of nsp are nsp1 - inhibition of host mRNA translation and affects signals from IFN, nsp2-survival signaling pathway of host cell, nsp3 - separation of the translated protein, nsp4-transmembrane domain 2 (TM2) and modifies ER membranes, nsp5-processing of polyprotein, nsp6-presumptive transmembrane domain, nsp7 and nsp8-increase the stable binding of nsp12 and template-primer 7SLRNA, nsp9-single stranded RNA-binding protein, nsp10- cap methylation of viral mRNAs, nsp12-RNA-dependent RNA polymerase (RdRp-for coronavirus replication/transcription), nsp13-binds with ATP and the zinc-binding domain to participate in the replication and transcription, and nsp14-proofreading exoribo-nuclease domain, nsp15-Mn(2+)-dependent endoribonu-clease activity and nsp16 - 2'-O-ribose methyltransferase and host mRNA splicing (Naqvi *et al*., 2020). NSP1 binds to 18S ribosomal RNA in the mRNA entry channel of the ribosome to interfere with the translation of mRNA. NSP8 and NSP9 bind to the 7SL RNA which located at the 'signal recognition particle' to disrupt protein trafficking to the cell membrane (Banerjee *et al*., 2020).

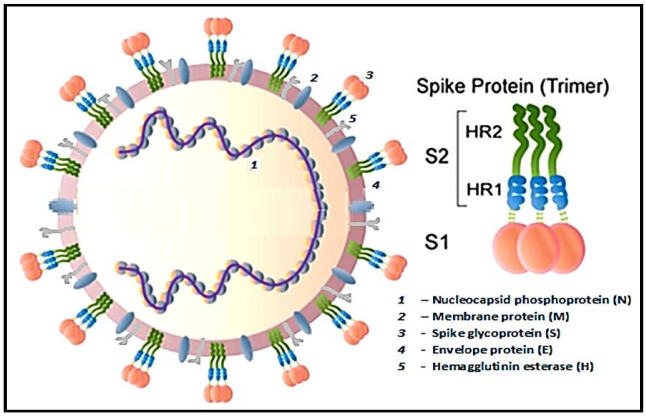

Figure 1: Structure of SARS-CoV-2 proteins.

2.1 Santigen

Santigen or spike glycoprotein plays important role in recognition and attachment of virus to the host cells. It is a homotrimer S1, S2 and transmembrane segments. S1 is consists of five antiparallely arranged beta strands linked by disulphide bonds referred as N-terminal domain (NTD) or receptor binding domain (RBD) binds to angiotensin-converting enzyme-02 (ACE-2) receptor present on the lungs, cardiovascular system, gut, kidneys, central nervous system, and adipose tissue of the human.

S2 subunit consists of a fusion peptide (FP), trimmers of heptad repeat 1(HR1), trimeric coiled - heptad repeat 2(HR2), central helix (CH), connector domain (CD), transmembrane domain (TM), and cytoplasmic tail (CT) which connects S1 domain with the virus. Proper folding of s-protein and neutralizing antibodies are associated with the glycans present on the S1 subunit and the whole domain protruded outside the virus during the time of infection (Wrapp *et al*., 2020). The HR1 and HR2 domains are linked by a hydrogen bond between lysine at 933 position in HR1 and asparagine at 1,192 position in HR2. Seventeen of the 20 residues of the ACE2 receptor interact with the RBD of SARS-CoV-2 with high affinity and forms a stable interaction the host (Figure 2).

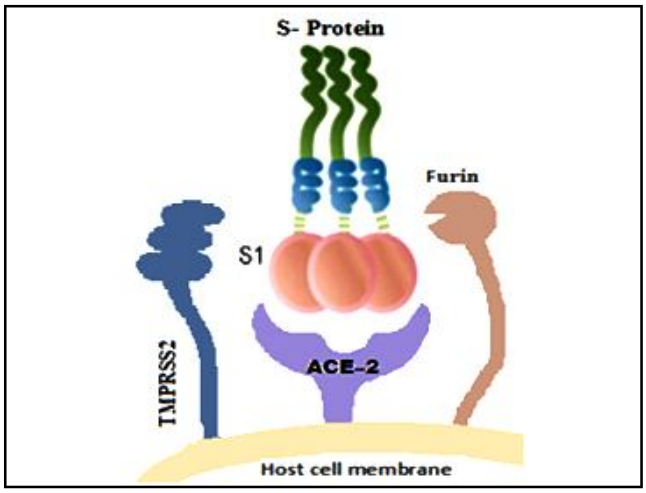

Figure 2: Interaction of S-protein with ACE-2 receptor on cell membrane.

Transmembrane serine protease-2 (TMPRSS2) is a surface protein expressed on endothelial cell that is involved in activation of spike protein in SARS-CoV-2 along with furin. To activate the S1 protein, cleaved by furin protease present near the ACE-2 receptor. It cleave linking region between S1 and S2 subunit on the human cell.The furin cleavage site includes P681, R682, R683, and A684. Loss of these cleavage site completely affects the pathogenesis of the virus by loss of binding function with ACE-2 receptor (Wang *et al*., 2020). Emma *et al*. (2020) described a rapid rise of SARS-CoV-2 infections due to the substitution at position S:677 polymorphism of the S gene that lead to 6 distinct variants suggest a selective advantage of the virus to escape from immune system and drugs.

2.2 The RNA-dependent RNA polymerase (RdRp)

The replication and transcription of SARS-CoV-2 is mediated through a combination of viral nsp and RNA dependent RNA polymerase in nsp 12, 7 and 8 complex. The nsp 12 has N-terminal end with a unique hairpin loop and C-terminal end which are linked by an interface domain.The RNA-dependent RNA polymerase domain consists of fingers subdomain, a palm subdomain, and a thumb subdomain (Wang *et al*., 2020) (Figure 3). The subunit nsp12 binds with the first turn of RNA between thumb domains and fingers domains. The active site of palm subdomain contains five nsp12 motifs A-E. The nsp8 extensions use the positively charged residues to interact with the RNA backbones. The nsp8 as the "sliding poles", slide on the protruding RNA to prevent RdRp from dissociating prematurely during replication. The triphosphate-binding site is

conserved. Residues D623, S682, and N691 are interacts with the 2'-OH group of triphosphate (NTP), making the RdRp special for the synthesis of RNA (Wang *et al*., 2020).

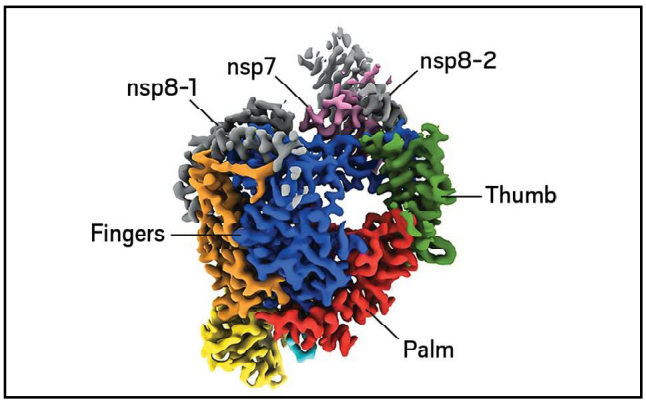

Figure 3: RNA-dependent RNA polymerase of SARS-CoV-2 (American Chemical Society).

2.3 The main protease

The main protease (M^{pro}) of SARS-CoV-2 plays a pivotal role in mediating the replication and transcription of viral gene. Inactive form of RdRp, nsp-13 and polyproteins (pp1a and pp1ab that is between nsp-4 and nsp-6) required for viral replication are cleaved by M^{pro} at eleven conserved sites. It belongs to the group cysteine protease with an active site contains cysteine and serine dyad and a water molecule. This has 99% of identity with bat coronavirus RaTG13 main protein. It is a homodimer of protomer A and protomer B, and each protomer contains 3 subdomains: domain I, domain II, and domain III. Domain II and domain III are linked by a loop and at the cleft between domain I and domain II lies the substrate-binding pocket (Jin *et al*., 2020).

Drugs used to control SARS-CoV-2 mainly aims at targets like viral spike protein (S), RNA-dependent RNA-polymerase (RdRp, nsp12), main protease, NTPase/helicase (nsp13) and papain-like protease (PL^{pro}, part of nsp3) which are essential for viral replication/ transcription and entirely different from human cellular component.

Table 1: SARS-CoV-2 variants and their adaptive advantages

Variant and origin	Spike protein substitutions	Adaptive advantages in the variants
B.1.526 (United States)	Spike: (L5F), T95I, D253G, (S477N), (E484K), D614G, (A701V)	• Reduced susceptibility to the combination of bamlanivimab and etesevimab monoclonal antibody treatment (fact sheet). • Reduced neutralization by convalescent and post-vaccination sera.
B.1.526.1 (New York)	Spike: D80G, 144, F157S, L452R, D614G, (T791I), (T859N), D950H	• Potential reduction in neutralization by monoclonal antibody treatments.
B.1.525 (United Kingdom / Nigeria) P.2 (Brazil)	Spike: A67V, 69/70, 144, E484K, D614G, Q677H, F888L Spike: E484K, (F565L), D614G, V1176F	• Potential reduction in neutralization by convalescent and post-vaccination sera.
B.1.1.7 (United Kingdom)	69/70, 144, (E484K), (S494P), N501Y, A570D, D614G, P681H, T716I, S982A, D1118H (K1191N)	• ~ 50% increased transmission (Davies *et al*., 2021). • Potential increased severity based on hospitalizations and case fatality rates (Horby *et al*., 2021). • No impact on treatment with monoclonal antibody treatments (Fact sheet). • Minimal impact on neutralization by convalescent and post-vaccination sera.
P.1(Japan/Brazil)	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027	• Significant decrease in susceptibility to the combination of baml-anivimab and etesevimab monoclonal antibody treatment, but other monoclonal antibody treatments are available (Fact sheet). • Reduced neutralization by convalescent and post-vaccination sera (Emary *et al*., 2021).
B.1.427 (United States - California)	L452R, D614G	• ~20% increased transmissibility (Deng, *et al*., 2021)· • Modest decrease in susceptibility to the combination of bamlanivimab and etesevimab; however, the clinical implications of this decrease are not known.Alternative monoclonal antibody treatments are available. • Reduced neutralization by convalescent and post-vaccination sera (Liu *et al*., 2021).
B.1.429 (United States - California)	S13I, W152C, L452R, D614G	• ~20% increased transmissibility (Deng *et al*., 2021). • Modest decrease in susceptibility to the combination of bamlanivimab and etesevimab; however, the clinical implications of this decrease are not known. Alternative monoclonal antibody treatments are available. • Reduced neutralization by convalescent and post-vaccination sera.

3. Variants of SARS-CoV-2

A variant is the organism having one or more mutations that differentiate it from other variants of a species. Globally, the SARS-CoV-2 variant having D614G mutation in the spike (S)-protein predominant strain. First recognized variant undergone S-D614 (aspartate) mutated to S-G614 (glutamine). Similar mutants of virus like particles of SARS-CoV-2 produced by mutation in M, N, E, and S proteins. This had increased infection rate of the mutant (Table 1). When mutation frequency of SARS-CoV-2 is calculated, it undergoes mutation at one or two single-nucleotide per genome in a month which is 50% of rate of mutation of flu virus and 25% of AIDS virus mutation rateand the overall the mutation rate is slow compared to other RNA viruses. A novel exoribonuclease (ExoN) coded by virus helps in correcting the errors during genome replication. Even though, the major adaptive mechanisms rendered by these mutations are:

- Specific genes to affect transmission, diagnostics, therapeutics, or host immune responses.
- Increased level of outbreak by antigenic variants.
- Significant reduction in vaccine effectiveness or very low vaccine-induced protection.
- Severe pathological condition (hyperoxia) and increased mortality.

3.1 S protein mutations in SARS-CoV-2 variants

Mutations in the S gene, region responsible for pathogenesis and normal function (such as the receptor binding domain (RBD) or furin cleavage site) or activation of the S protein, are of the greatest interest. These mutations may provide an avenue for the virus to escape from immunity to the original SARS-CoV-2 strain. First reported mutation in S-protein at site D614G site and the mutant has increased infectivity by assembling more functional S protein into the virion (Zhang *et al*., 2020). D614G is a substitutional mutation at 614 position of S protein by replacement of aspartate (D) with glycine (G) that enhanced viral replication in human lung epithelial cells and primary human airway tissues by increasing infectivity and stability of virons (Plante *et al*., 2021). Novel variant of SARS-CoV-2 is B.1.1.7, commonly referred to the U.K. strain, variant B.1.351 known as 501Y.V2 or the South African variant, P.1., is known as 501Y.V3 or the Brazilian variant; B.1.427 and B.1.429 (Zhang *et al*., 2021), CAL.20C or the California variant recognized as B.1.526, or the New York variant and multiple lineages of variants that contain mutations at amino acid position 677 (CDC, 2021). SARS-CoV-2 variants have undergone major mutation in cleavage site, in B.1.1.7 substituted from proline (P) to histidine (H) at position 681, B.1.351 and B.1.526 both have undergone an alanine (A) to valine (V) substitution next to the furin cleavage site at 701. A base substitution recorded at the position L452R in B.1.526.1, B.1.427, and B.1.429 also another substitution of at E484K is present in B.1.525, P.2, P.1, and B.1.351, but only some strains of B.1.526 and B.1.1.7. B.1.1.7 variant is associated with increased risk of death compared to other variants (Weisblum *et al*., 2020) (Figure 4). Similar amino acid changes in the spike protein of B.1.1.7, B.1.351, P.1, B.1.427/B.1.429 and B.1.526 SARS-CoV-2 variants were recorded.

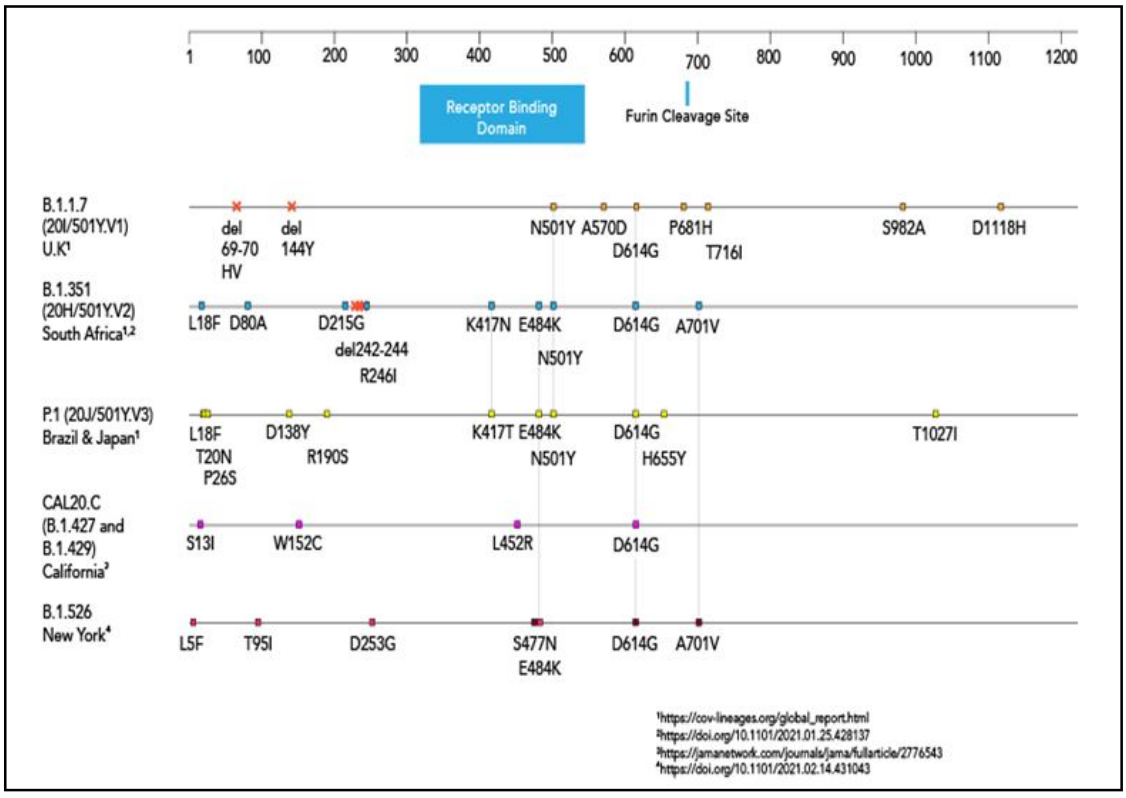

Figure 4: Amino acid changes in the spike (S) protein of SARS-CoV-2 variants.

Source: American Society for Microbiology (Ashley Hagen, 2021).

Alteration in antigenicity attributed by the deletion of amino acids in the position 69 and 70 mutation in the N-terminal S_1 subunit of S protein cause a conformational change in the spike protein and the emergence of B.1.1.7 variant. Deletion of amino acid 144 in B.1.1.7 and amino acids 242-244 in B.1.351 decreased the binding ability of the virus with neutralizing antibodies (By Kevin *et al.*, 2021).

Substitution of aspartate with glycine at position 253 that appears in B.1.526, helps to escape from monoclonal antibodies against the NTD, and L18F, a leucine (L) to phenylalanine (F) substitution at position 18 in P.1 and R246I variants, an arginine (R) to isoleucine (I) substituted at position 246 in B.1.351.

B.1.427/B.1.429 and B.1.526 alsorecognized to have amino acid substitutions in the NTD (Ashley Hagen, 2021).

3.2 RBD site variants

The receptor binding domain (RBD) of S protein is comprised of amino acids 319-541 and it directly binds to ACE2 receptors. Therefore, mutations in this portion affects the antigenicity. The variants B.1.1.7, B.1.351 and P.1 have replacement of asparagine (N) with tyrosine (Y) at position 501 of the RBD, which results in reduced antibody production and binding to RBD (Vipin Vashistha, 2021).

Variants B.1.351 and P.1 have RBD mutations in common, K417N or K417T, a lysine (K) to asparagine (N) or threonine (T) substitution at position 417, and E484K, a glutamate (E) to lysine (K) substitution at position 484, that increases the affinity of RBD for ACE2, resistance to antibody neutralization, and antibody mediated therapy. The California strain B.1.427 and B.1.429, both contain the same 3 S gene mutations. One of these, L452R, is a substitution that replaces leucine (L) with arginine (R) at position 452 and increased the affinity of RBD and cause severe infection (Meredith Wadman, 2021).

3.3 Furin cleavage site variants

Mutation in furin cleavage site of S protein affects activation of virus to attach with the host cell. B.1.1.7 variant has a proline (P) to histidine (H) substitution at position 681 near the furin cleavage site.

B.1.351 and B.1.526 have an alanine (A) to valine (V) substitution at position 701 (A701V) that is still of which are of unknown function. Rapid rise in mutation at furin cleavage site resulted in emergence of 6 distinct variants having substitutional mutation at 677 position of furin cleavage site of s protein. Histine at 677th position substituted with glutamine (Q677H) in B.1.2, B.1.234, B.1.1.220 and B.1.1.222 and other two variant showed presence of proline substituted glutamine at 677 position (Q677P). Increasing spread rate of these variants and infectivity proven that these mutations cause high potential functional relevance in cell entry and deliberate an advantage in spread or transmission (Hodcroft *et al.*, 2021).

3.4 C-terminus variants

S_2 helps in binding of spike protein with host cell membrane also undergoes multiple mutation effects in efficient binding with host or of unknown function. The variants B.1.1.7 and P.1 have multiple mutations in the C-terminal domain of unknown significance.

4. Mechanism of pathogenesis by SARS-CoV-2

Biopsy of SARS-Cov-2 infected patients showed diffused alveolar sac and wall with the formation of hyaline membranes, mononuclear cells, and infiltration of macrophages in the filtrating air spaces. Presence of large number of viral particles were observed in the bronchial and type 2 alveolar epithelial cells under electron microscopy. In addition, spleen atrophy, lymph node necrosis, focal haemorrhage in the kidney, enlarged liver with inflammatory cell infiltration, oedema, and scattered degeneration of the neurons in the brain recorded in severely ill patients by autopsy suggesting multiple organ dysfunction (China National Health Commision, 2020 and Yao *et al.*, 2020). However, there are no reports about broad dissemination of the viral particles by autopsy. Gastrointestinal tract and kidney cells expressing ACE-2 receptors also infected by SARS-CoV-2.

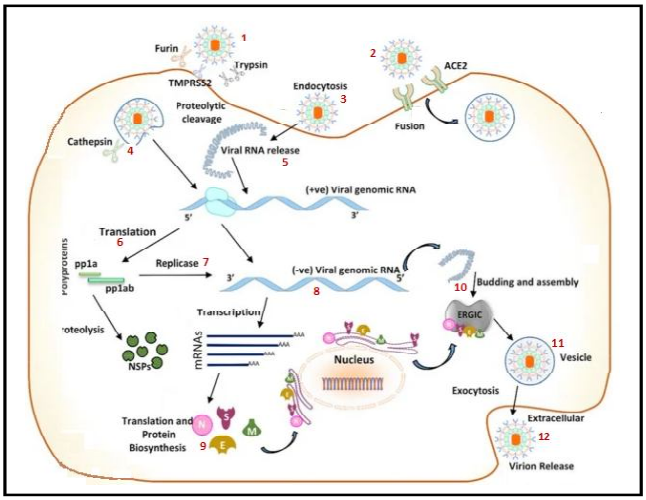

Figure 5: Life cycle of SARS-CoV-2 in human cell.
***Source:** Yesudhas *et al.*, 2021 (CC).

The S protein present in the membrane of SARS-CoV-2 recognizes the host ACE-2 receptor and binds to the receptor by activation of the spike protein by cleavage between S1 and S2 domain. TMPRSS2 is a serine protease present frequently in alveolar epithelial type II cells, it primes the spike-domain (S) of SARS-CoV-2 by cleaving as the S1/S2 sites. The spike protein is cleaved at S20 by TMPRSS2, results in the fusion of membranes with virus. After fusion with the host cell entry of viral RNA into the host cell by the clathrin-dependent, and/or clathrin-independent endocytosis (Wang *et al.*, 2008) (Figure 5). By using the RNA dependent RNA polymerase enzyme, viral genome undergoes replication and transcribed to two structural proteins and polyproteins in the cytoplasm of the host cell. Simultaneously, host cell activates coordinated immune response through innate and adaptive immune response. In endosome, membrane specific pattern recognition receptors (PRR), such as toll-like receptor (TLR3, TLR8, TLR7, and TLR9) or the cytosolic RNA sensor, RIG-I/MDA5 or the secretory type PRR like mannose-binding lectin (MBL) and C-reactive protein (CRP) recognize viral RNA by pathogen-associated molecular patterns (PAMPs) (Perlman and Netland, 2009). Interferon (IFN-1) type I activate a potent innate immune response and adaptive immune response. A complex signaling cascade initiated by recruiting adaptor proteins such as mitochondrial antiviral-signaling protein (MAVS), IFN-β (TRIF), and stimulator of interferon genes protein (STING) and activate downstream cascades

molecules, like adaptor molecule MyD88. Stimulatory molecules further activates transcription factors like nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF3) and helps in nuclear translocation. In the nuclei, these transcription factors induce the production of type I interferons (IFN-α/β) and a plethora of pro-inflammatory cytokines especially IL-6 (Li *et al*., 2020). Type I IFN suppress viral replication and promote phagocytosis of antigens by macrophage, as well as NK cells mediated restriction of infected cells (Zhou *et al*., 2020).

Generally, Th1 mediated immune response plays a predominant role in T cell responses that depend upon the presence of APC (antigen presenting cells) mediated cytokine microenvironment. $CD8^+$ cytotoxic T cells (CTLs) secrete granzymes, perforin, and IFN-γ lyse the virus infected host cell and eradicate it. $CD4^+$ Helper T cells induce the T cytotoxic cells and on the other hand, B-cell mediated humoral immune response. Through, the clonal propagation method induce the production of memory B cells and plasma cell against the each viral antigen to produce neutralizing antibody and prevent from further infection (Figure 5). Similar to SARS and MERS infection an elevated level of chemokines and plasma cytokines such as interleukins (IL-1, IL-2, IL-4, IL-7, IL-10, IL-12, IL-13, and IL-17), IP-10, macrophage colony-stimulating factor (MCSF), MCP-1, GCSF, hepatocyte growth factor (HGF), IFN-β, MIP-1α, and TNF-α, *etc*., are expressed from the virus infected cells referred as lymphopenia or cytokine storm (Moore and June, 2020) (Figure 6).

Cytokine storm is due to the deregulated and excessive immune responses that cause damage to the host body. The cytokine storm cause the severe cytolysis of organs (air sac/lung/kidney/spleen/intestinal tract) and further viral infection such as acute respiratory distress syndrome (ARDS), pneumonitis, respiratory failure, sepsis shock, organ failure, and potentially death. Severity of COVID-19 in patients cause decrease in the number of circulating B cells, CD8 + cells, CD4 + cells, natural killers (NK) cells, and a decrease in eosinophils, monocytes, and basophils (Zhou *et al*., 2020). Apoptosis of endothelial cells and epithelial cells damages the pulmonary microvascular and alveolar epithelial cell barriers and causes vascular leakage and alveolar edema, eventually leading to hypoxia (oxygen deprived condition in the tissues). In severe case of ARDS innate immunity cause induction of hypoxia inducible factor-1α (HIF-1α) that cause up-regulation of vascular endothelial growth factor (VEGF). These factor induce the improper immune response by nonspecific inflammatory cell infiltration with increased vascular permeability leads to autophagy recruitment (Jahani *et al*., 2020). Autophagic recruitment cause blood (hypoxemia) and tissue (hypoxia) oxygenation plays major role in inflammation, capillary damage, organ failure and death due to collapse of lungs and cardiac arrest (Ostergaard, 2021). The cytokine storm is also a cause of death in ICU patients by increase the level of liver enzymes in gastrointestinal tract and creatinine levels in kidney that lead to extrapulmonary multiple-organ failure (Ye *et al*., 2020).

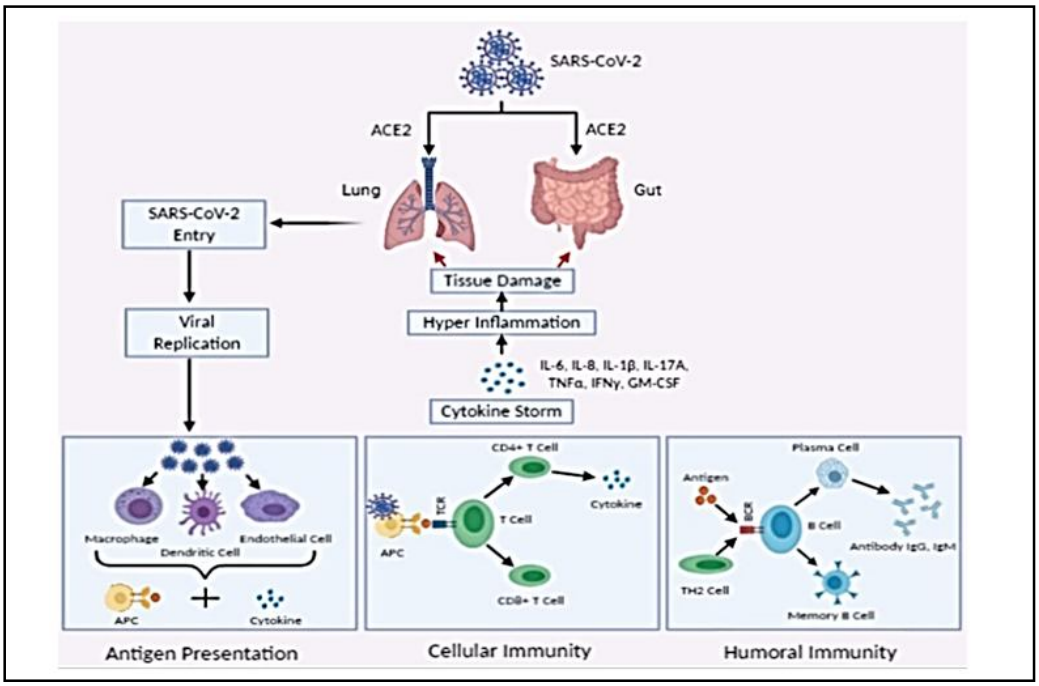

Figure 6: Immune response to SARS-CoV-2 infection in human body (Chatterjee *et al*., 2020).

In vitro cell experiments report that delayed release of cytokines and chemokines occurs in respiratory epithelial cells, dendritic cells (DCs), and macrophages at the early stage of SARS-CoV-2 infection completely arrest the host antiviral response in the host cells and induce inflammation. Later, the cells secrete low levels of the antiviral factors such as interferons (IFNs) and high levels of proinflammatory cytokines (interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)) and chemokines (C-C motif chemokine ligand (CCL-2, CCL-3, and CCL-5).

Delayed release of IFNs in the early stages of SARS-CoV and MERS-CoV infection hinders the body's antiviral response (Channappanavar *et al*., 2019). Accelerated cytokine storm was also recognized and recorded in animal models such as mice, primates. In endothelial

cell line model the production of IFN-αβ and IFN-γ induced inflammatory cell infiltration through mechanisms involving Fas-Fas ligand (FasL) or **trail**-death receptor 5 (DR5) and cause the apoptosis of airway and alveolar epithelial cells (Rodrigue *et al*., 2014).

5. Treatment

Treatment for the COVID-19 infection developed by following two strategies: aimed to restrict or block the activity of the specific viral component or modulating host immune response. Again host immune response modulated in two different ways for treatment: boosting immune response during initial stage of the infection and suppress the immune response during later/ high inflammatory stage of the infection. Many of the drugs are primarily targeted towards inactivation of virus specific components such as spike protein, nsp, RdRp, nucleocapsid phosphoprotein, RBP, inhibitors to 3-chymotrypsin like protease (3CLpro), *etc*. Different drug components are already in use against SARS-CoV/ newly developed drugs/ drugs under development are targeted towards the SARS-CoV-2. The following table brief on drug candidates and their function (Table 2).

Table 2: Drugs used for treatment of SARS-CoV-2 infection

Drug of choice	Mechanism of action	Outcome	References
IFN-λ	Activates epithelial cells and reduces the mononuclear macrophage-mediated proinflammatory activity of IFN-αβinhibits the recruitment of neutrophils to the sites of inflammation activates the antiviral genes in epithelial cells	Early administration reducing viral load	Davidson *et al*., 2016
Corticosteroid (Dexamethasone)	Suppress inflammation	Treatment during initial period of infection reduced the mortality rate. But later cases caused increased severity of infection large doses delay the clearance of virus due to immunosuppression	Auyeung *et al*., 2005; Yam *et al*., 2007
Passive immunization with IgG (casuruvumab/ imdevimab)	immune substitution and immunomodulation	Effective on original strain neutralization but not on variants	Shakoory *et al*., 2016; Fischer *et al*., 2020
Blockers of VEGP (*e.g*., bevacizumab)	Suppress the expression of VEGF and reduce vascular permeability	Under clinical trials	Leah Shaffer, 2020
Blockers of IL-1 (*e.g*., anakinra)	Reduce the cytokine storm	Improved survival rate of patients with severe symptoms	Biggioggero *et al*., 2018
Blockers of IL-6 (*e.g*., tocilizumab)	Reduce the cytokine storm in severely infected persons	Improvise the severely inflamed organs	Tanaka *et al*., 2016
Blockers of TNF	Blocks the receptors to bind with TNF	Recommended during later stages of infection. Prevent from lung damage. Still need to explore	McDermott *et al*., 2016
Inhibitor of IFN-αβ	Prevent frominflammation	Administrated only later stages of the infection to reduce excessive inflammatory responses	Davidson *et al*., 2015
Chloroquine	Inhibits the production of TNF and IL-6	suppress the cytokine storm	Gao *et al*., 2020
Hydroxychloroquine	Reduces the viral load during early stage of infection	Effect is enhanced by Azithromycin administration	Gautret, *et al*., 2020
Ulinastatin	Naturalanti-inflammatory	Protects the vascular endothelium Reduce cytokine storm	Wang *et al*., 2019
Inhibitors of oxidised phospholipids (OxPL)	Reduces the production of inflammatory chemokines and cytokines	Reduce the rate of acute lung injury	Imai *et al*., 2008
Antagonist to sphingosine - 1 phosphate receptor	Prevent the synthesis of cytokines	Reduces proinflammatory responses in lungs	Walsh *et al*., 2011; Teijaro *et al*., 2011

Mesenchymal stem cell therapy	Inhibit abnormal activation of Th1 cells and innate immune cells	Alleviate ARDS	Uccelli *et al*., 2015
Blood dialysis	Block cytokine storm and reduce the risk of inflammation	Used to treat severely ill patients	Xu. K., *et al*., 2020
Inhibitors of macrophage recruitment (siRNA)	Completely reduces the inflammation	Not clinically proven in COVID-19 patients/ animal models	Leuschner *et al*., 2015
Ivermectin	Binding and destabilizing cell transport proteins	Still studies are going on.	Leah Shaffer, 2020
Remdesivir	Inhibitor of RdRp	Reduces the viral load in the lung	Wang *et al*., 2020
Lopinavir	Inhibitor of viral protease -3-chymotrypsin -like protease	Used along with ritonavir	Chen *et al*., 2006
Famotidine	May bind to papain-like protease	Prevent the entry of virus into cell	Freedberg *et al*., 2020
Nafamostat	Serine protease inhibitors (TMPRSS2)	Inhibit viral cell entry	Markus Hoffmann *et al*., 2010
Blood thinners/ anticoagulants	Heparin or warfarin. Prevent coagulation of blood by SARS-CoV-2	Reduced the risk of blood coagulation. Still research need to be done	COVID-19 treatment guidelines panel

6. Probiotics in control of SARS-CoV-2

Though, several drugs and natural components used for the treatment of COVID-19, the major role of human natural flora, friendly bacteria - probiotics their role in COVID -19 infection need to be elucidated. A potential therapy to block the multiplication of live organisms by other living organisms can be used to ensure continuous monitoring and regulation in the entry of pathogens into the body. The gastrointestinal tract of the human body includes bacteria, viruses, and fungi (Shi *et al*., 2017). The number of organisms varies from 10^4 to 10^5 CFU/ millimeter of the small intestine and 10^{11} CFU/ gram of lumen content of the colon (Thomas *et al*., 2017). Major gut probiotic microbiome includes *Lactobacillus acidophilus*, *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. delbrueckii* subsp. *bulgaricus*, *L. brevis*, *L. johnsonii*, *L. plantarum, L. fermentum, Saccharomyces boulardii*, *Bifidobacterium bifidum, Lactococcus lactis, etc*. The probiotic microbial population in the gut comprises 90% of *Firmicutes* and *Bacteroidetes* followed by other *Proteobacteria* and *Actinobacteria* (Rinninella *et al*., 2019).

Previous clinical studies reported that probiotics prevent bacterial and viral infections, including gastroenteritis, sepsis, and respiratory tract infections especially highly active in controlling 80% of respiratory tract infections caused by respiratory syncytial virus, rhinoviruses and influenza viruses (Baud *et al*., 2020).The major proposed mechanism used to control SARS-CoV-2 by the probiotics includes (Al Kassaa *et al*., 2014);

i. Interaction of probiotics directly inhibit virus lodging in the host system
ii. Production of various antiviral metabolites
iii. Immunomodulation of host

6.1 Competitive inhibition

Enriching the gut with probiotic population competitively inhibits the virus loading and multiplication in the gut. The virus propagates and establishes in the host gut when dysbiosis of probiotic population. Administration of probiotic organisms cause gut mediated-pulmonary immunity in virus-infected host system and reclaim health (Gohil *et al*., 2021). Antagonism of probiotics on the virus and its immunomodulatory function ameliorated by the mixed consumption of probiotics with prebiotics (Olaimat *et al*., 2020). Probiotics reported to competitively inhibit HIV adsorption to the host by the production of sulfated exopolysaccharides and by surfactin produced by *Bacillus subtilis* OKB105 inhibits adsorption by transmissible gastroenteritis virus. Well-known entry and attachment site of the SARS-CoV-2 manipulated by the presence of well flourished probiotic community and has attracted as a potential therapeutic target for a number of diseases. In COVID-19 infection, probiotics exert pressure on the expression of angiotensin converting enzyme 2 (ACE-2) in the small bowel and colon and prevent gut inflammation (Segal *et al*., 2020). Establishment of SARS-COV-2 in the gut region reported replacing the beneficial probiotic bacteria (*Eubacterium ventriosum, Faecalibacterium prausnitzii, etc*.) with opportunistic pathogens (*Clostridium hathewayi, Actinomyces isrellii, Bacteroides nordii*). Further complications associated with viral infections enhanced by these opportunistic pathogens. Other physical factors which complicate the health of COVID-19 patients include old age, heart diseases, obesity, hypertension and diabetes mellitus (uptake of associated medicines) leads todysbiosis of probiotics, which contribute to the severity of infection (Mak *et al*., 2020).

6.2 Antiviral metabolites

Probiotic organisms produce various metabolites that inhibit the establishment of viruses in the host. Biosurfactants are surface-active molecules that disrupt the lipid envelope of the virus. Similarly, fermentation end products such as organic acids (butyric acid/ lactic acid) produced by the probiotic organism prevent the actuality of the virus in the gut and down-regulate the intestinal permeability by the virus. Production of butyric acid energies the intestinal colonocyte which in turn constricts the integrity at tight junction where SARS-CoV-2 attachment to the gut epithelial cells and colonization becomes challenging (Baud *et al*., 2020). Metabolites from *Lactobacillus plantarum* such as plantaricin, lactic

acid, acetic acid, and gamma-aminobutyric acid showed antiviral activity by binding with the virus and prevent it from associating with host system. Molecular docking studies reported that complete detention of binding of RBD and spike protein with ACE-2 by the probiotic metabolite plantaricin BN, plantaricin JLA-9, and plantaricin D. The maximum binding energies obtained with plantaricin W recorded as -14.64, 11.1 and 12.68 for polymerase, RBD and ACE2. Plantaricin W, D, and JLA-9 were able to block the residues (THR556, ALA558) surrounding the catalytic site (VAL557) of RdRp in SARS-CoV-2. So, the potential of plantaricin to control SARS-CoV-2 established with *in silico* model (Anwar *et al.*, 2020).

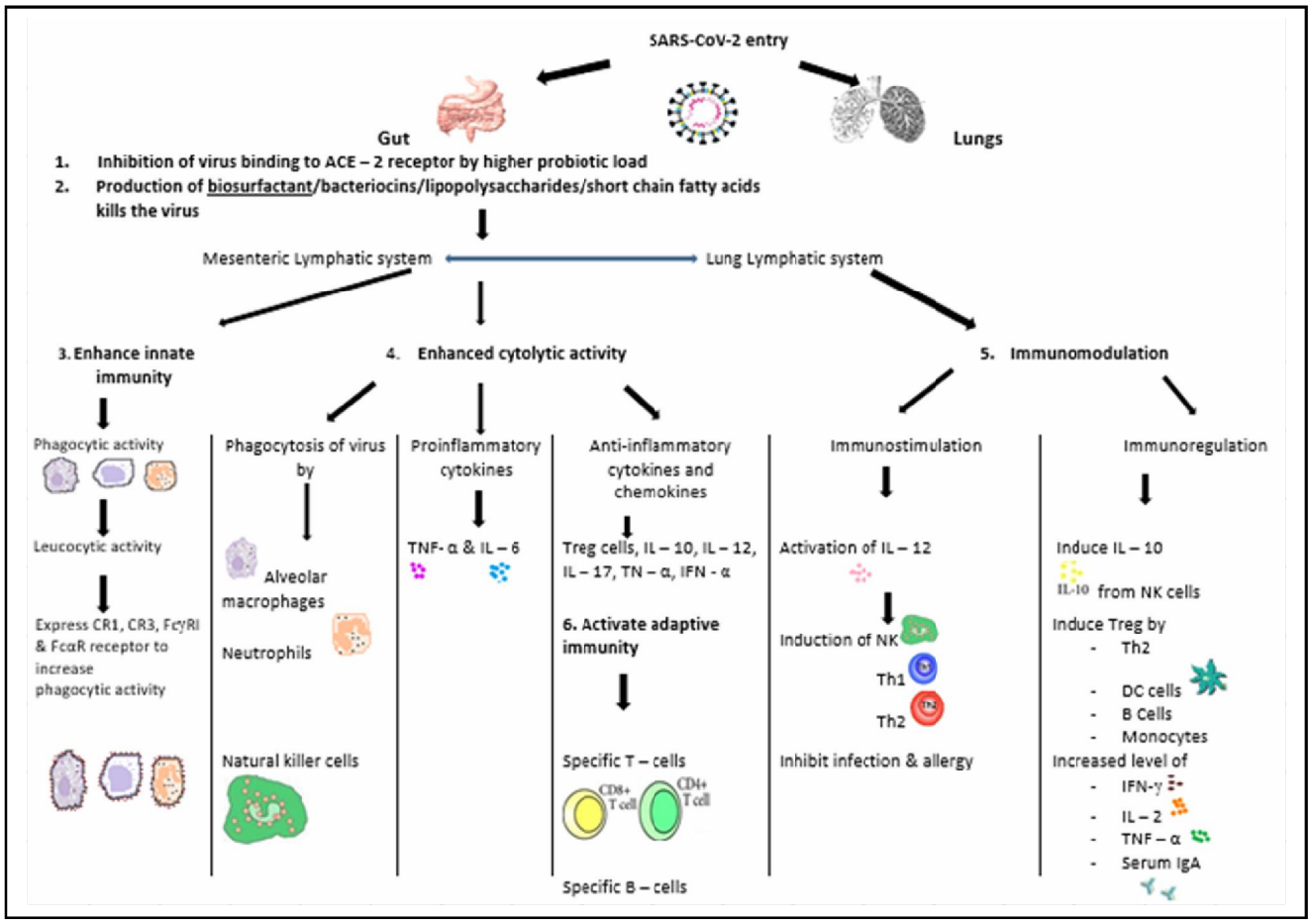

Figure 7: Immune mechanism to control SARS-CoV-2 by probiotics.

6.3 Immunomodulation

The probiotics are proven for their immunomodulatory effect upon viral infections in the human. Experimental data confirmed the increase in T helper cell-mediated immune response in influenza virus-infected parenchymal cells by *Lactobacillus* sp., and *Bifidobacterium* sp. Induction of phagocytosis of virus infected cells and increased innate immunity by the production of IL-4, IL-10, and IL-6 recorded in bronchoalveolar lavage by the *L. rhamnosus* and *B. lactis*. Clinical trials on the effect of probiotics in the management of SARS, MERS and SARS-CoV-2 need to be explored (Sundararaman *et al*., 2020). The probiotic organisms modulate the gut and lung by entering into the lymphatic system. From the mesenchyme lymphatic system moves to the lung lymphatic system and modulates the immunity of the host. Actually, major sites of multiplication of SARS-CoV-2 in the host are the lung and gut. Immunomodulation in these regions certainly affects the pathogenicity of the virus. In the early stage of viral infection, inflammation and poor immune response result insevere pathological condition, in later stages excess of the immune response or cytokine storm leads to a sudden collapse in the host. Both immunological conditions are well maintained by the probiotic immunomodulatory function. First of all, probiotics enhance innate immunity by the cytolytic activity of macrophages, monocytes, neutrophils, natural killer cells, and allied cytokines to destroy the virus-infected cells. Secondly, by immunostimulatory task, activates the specific T-cells to induce cytotoxic activity and inflammatory mediators. Next by immunoregulation, triggers the specific neutralizing antibody production, meantime regulates the excess/uncontrolled expression of cytokines by treg mediators such as Th2, dendritic cells, and monocytes. Also regulate the inflammation, induction of IFN- g enhance B cell-mediated adaptive immunity and complement fixing antibodies (Figure 7). By the induction of treg mediators such as IL-25 migration of lymphoid cells from the gut to the lungs to dislodgement of pathogens recorded (Huang *et al*., 2018). Bifidobacteria recognized to have the potential to ameliorate the viral cytokine storm through regulated expression of pro and anti-inflammatory cytokines (Hüseyin *et al*., 2020).

The theoretical studies and experimental data of other viral infections concluded that probiotic bacteria may catalyze immune homeostasis during coronavirus-infections (Singh and Rao, 2021). Thus, the probiotics functions as live neutraceutical and life-saving immunobiotic from infections caused by SARS-CoV-2 and its variants. Also recommended that in absence of efficient treatment strategy, probiotics also used parallel with other treatment method (Gohil *et al.*, 2021). Still, clinical studies on the mechanism of action of probiotics *in vivo* condition needs to be established in COVID-19 patients.

7. Future prospects

SARS-CoV-2 is the variant of the SARS virus, drastically affecting human society and the economy. The virus is mutating at a faster rate and adapting to escape from drugs that are used for treatment. Similarly, already developed vaccines reported to have less efficacy in combating the viral variants. The 86% efficiency of Covishield vaccine towards the wild type strain reduced to less than 63% on B.1.1.7 variant, 5% reduction in the efficacy of Novavax vaccine reported on variants (89%) versus non-variant strain (84%), and Astra Zeneca vaccine efficacy reduced from 84%on non-variant to 74% on variant strains. A ten-fold efficacy reduction in Pfizer and Moderna vaccine, 40% reduction of efficacy of Novavox against South African variant reported. Moreover, in the city of Manaus, Brazil about 70% of the persons already infected by the wild type strain of SARS-CoV-2 and acquired natural immunity toward the virus. But, records shows that again vaccinated individuals infected by the variant of SARS-CoV-2. These study results envisaged the inefficacy of natural immunity and acquired immunity (through vaccines) to prevent infections caused by variants. As the number of vaccines and drugs are developing, parallel variants also emerging. So, a live immune booster that continuously enhances the host immunity and competitively inhibits the entry of the virus can assure the remedy to viral infection. Also, pathological viruses cannot be eradicated by a single mechanism such as by administration of drug or vaccine as the virus is showing phase shift. An immunomodulator along with drugs or vaccines can complement the activity and eradicate the virus and its variants. An exploratory *in vivo* studies required to address the full proof of concept on the immunomodulatory effect of probiotic to control SARS-CoV-2.

Conflict of interest

The authors declare that there are no conflicts of interest relevant to this article.

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