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Probiotics and the reduction of SARS-CoV-2 infection through regulation of host cell calcium dynamics

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ABSTRACT

Calcium is a secondary messenger that interacts with several cellular proteins, regulates various physiological processes, and plays a role in diseases such as viral infections. Next-generation probiotics and live biotherapeutic products are linked to the regulation of intracellular calcium levels. Some viruses can manipulate calcium channels, pumps, and membrane receptors to alter calcium influx and promote virion production and release. In this study, we examined the use of bacteria for the prevention and treatment of viral diseases, such as coronavirus of 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Vaccination programs have helped reduce disease severity; however, there is still a lack of well-recognized drug regimens for the clinical management of COVID-19. SARS-CoV-2 interacts with the host cell calcium (Ca^{2+}), manipulates proteins, and disrupts Ca^{2+} homeostasis. This article explores how viruses exploit, create, or exacerbate calcium imbalances, and the potential role of probiotics in mitigating viral infections by modulating calcium signaling. Pharmacological strategies have been developed to prevent viral replication and block the calcium channels that serve as viral receptors. Alternatively, probiotics may interact with cellular calcium influx, such as *Lactobacillus* spp. The interaction between *Akkermansia muciniphila* and cellular calcium homeostasis is evident. A scientific basis for using probiotics to manipulate calcium channel activity needs to be established for the treatment and prevention of viral diseases while maintaining calcium homeostasis. In this review article, we discuss how intracellular calcium signaling can affect viral replication and explore the potential therapeutic benefits of probiotics.

1. Introduction

Viruses have various forms of interactions with their hosts, which can provide advantages and even contribute to their survival. Viruses have a significant impact on safeguarding human health and evolution by contributing genes and mitigating the symptoms of diseases caused by other viruses [1]. Phage therapy is gaining attention as a bacteria-restricted virus that can potentially combat multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacteria [2,3]. However, opportunistic viruses have become a concern for society because of their association with epidemics and economic losses, such as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [4].

Methods for treating and preventing coronavirus disease of 2019 (COVID-19) have been developed as knowledge about the epidemiology and pathology of SARS-CoV-2 continues to increase [5]. However, viruses with properties that benefit the functions and cellular products of the host have been selected, allowing for more efficient viral replication [6,7]. Healthcare professionals have been working to address the challenge of developing new vaccines more quickly because genetic mutations can introduce uncertainties [8].

Research on the gut microbiota has revealed a wide range of probiotic strains, leading to an increase in the number of microorganisms that can be used in pharmaceutical applications and expanding the options for conventional probiotics. Next-generation probiotics (NGP) are microorganisms that meet the standard definition of probiotics and have not been previously utilized for health promotion. NGPs also align with

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the US Food and Drug Administration (FDA) definition of live biotherapeutic products (LBPs), which are biological products containing live organisms, such as bacteria, used for the prevention, treatment, or cure of human diseases or conditions [9,10].

LBP has various applications, including the treatment of gastrointestinal conditions such as antibiotic-related and cancer-related diarrhea; dental disorders such as cavities and periodontal diseases; allergies such as allergic rhinitis, asthma, and atopic dermatitis; conditions related to the gut and brain such as schizophrenia, depression, and Alzheimer's disease; and other conditions such as acne, hepatic encephalitis, and urinary tract infections [11]. The European Pharmacopoeia defines the terms LBP and NGP similarly but excludes fecal microbiota transplantation and gene therapy agents from the NGP category [12].

The use of probiotics NGP and LBP shows promise for exploring new approaches for the treatment and prevention of calcium-related viral diseases [13]. According to Kan et al. (2023), a strong correlation between intracellular calcium levels and viral infection was also found in an animal study comparing intracellular calcium and probiotic treatments. They observed lower intracellular calcium levels in pigs treated with *Lactiplantibacillus plantarum*. Although this study did not evaluate calcium mechanisms in human cells, there are data that demonstrate great similarities between pigs and humans [14–16], including anatomical size and structure, physiology, immunology, and genome, thus enhancing their potential as models for humans [14].

The present study aimed to demonstrate that molecules produced during the metabolism of LBP, probiotics, and second-generation probiotics play a role in cell signaling pathways that maintain intracellular calcium balance. Therefore, these molecules could potentially be targeted to prevent viral infections. Nevertheless, there is a scarcity of research establishing a correlation between viral and bacterial cycles and calcium metabolism. This correlation is crucial for developing effective probiotic combinations to mitigate viral diseases such as COVID-19. Therefore, we conducted a survey of the use of drugs that affect calcium homeostasis in hosts to treat viral diseases. We then applied these methods to analyze the interactions between probiotics, NGP, LBP, viruses, and intracellular calcium homeostasis. Several hypotheses have been proposed to study new forms of medication for viral diseases.

2. Material and methods

Data collection was performed using bibliographic research carried out using search engines for scientific articles, including PubMed and Google Scholar. The major keywords were established "Live Biotherapeutics Products," "Probiotics," "Next-Generation Probiotics," "Bifidobacterium," "Lactobacillus," "Calcium," "Calcium Homeostasis," "Channel Blocker," "Virus," "COVID-19" and "SARS-CoV-2."

Articles written in English and published predominantly between 2018 and 2023 were included. Certain older articles were included in this review because of their significance in providing context for the study.

Articles were selected based on the content of current methods for controlling viral diseases and the use of medicines that control cellular calcium homeostasis. Articles were also chosen if they could create a link between microorganisms and viral activity inside the cell.

Once the data were selected and organized, an analysis was performed to link aspects of the relationship between viruses, intracellular calcium homeostasis, and information on the effects of probiotics, NGP, and LBP on host health. This evaluation was based on the possible benefits of these regulatory agents in the intervention of the mismatch in calcium levels caused by viruses inside the cell. Subsequently, ideas were proposed on how this methodology could be used as an alternative for controlling pathogenic viruses in humans.

The figures were created using BioRender.com.

3. Calcium transport in mammalian cells

In mammalian cells, Ca^{2+} functions as an intercellular secondary messenger and plays a role in various cellular processes, including protein transport, endosome formation, and synaptic plasticity. The intracellular medium has two main sources of Ca^{2+}: stored Ca^{2+} in the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR), and Ca^{2+} in the extracellular medium [17]. Lysosomal Ca^{2+} plays crucial roles in various cellular processes, including signal transduction, vesicular trafficking, autophagy, and exocytosis [18]. The plasma membrane contains various channels and receptors that facilitate the movement of calcium ions (Ca^{2+}) in and out of the cell. These include the transient Ca^{2+}-ATPase pumps, receptor potential channels, Na^{+}/Ca^{2+} exchangers, store-operated channels, and purinergic receptors [19]. The regulation of intracellular calcium levels is essential for the promotion of various cellular processes [20]. The plasma membrane controls the entry of Ca^{2+} from the extracellular space to the intracellular medium through three types of calcium channels: voltage-gated calcium channels, which are activated by membrane depolarization; receptor-operated calcium channels, which are stimulated by agonist substances or intracellular messengers; and store-operated calcium channels, which are activated when stored Ca^{2+} levels are depleted [6].

The regulation of calcium release into the intracellular environment is mediated by IP_3 receptors (IP_3R) and ryanodine receptors (RyRs). Triphosphate-1,4,5-inositol (IP_3) is responsible for transports Ca^{2+} into the internal medium [17].

The structures responsible for transporting Ca^{2+} from the internal medium to the extracellular medium, ER, or SR are the Calcium Pumps found in the Plasma Membrane (PMCA), reticulum (SERCA), and sodium-calcium exchanger (NCX). The functions of these structures are crucial for maintaining the intracellular calcium balance [6].

Therefore, interventions in the mechanisms regulating intracellular ion levels can affect viral reproduction [7]. There are methods to prevent viruses from using cellular structures with calcium channel blockers (CCBs). CCBs are classified into two groups based on their physiological effect: dihydropyridines, such as amlodipine, nifedipine, felodipine and nicardipine; and non-dihydropyridines, such as verapamil and diltiazem. Dihydropyridines primarily target peripheral vascular smooth muscle cells, while non-dihydropyridines are more selective for cardiomyocytes. These drugs act by blocking the alpha-1 subunit of L-type voltage-gated calcium channels, leading to prevention of calcium influx [21]. However, they may have potential side effects such as cardiac ischemia, cardiac arrhythmias, atherosclerosis, hypertension, and long-term complications [22,23]. Current drugs have not yielded positive results. Therefore, the pharmaceutical industry has conducted research on natural alternatives to meet the demand for treatments that do not negatively affect patients. One such alternative is Bifendate, an intermediate compound isolated from *Schisandra chinensis*, is used in traditional Chinese medicine (Turcz.) Baill exhibits antihepatitis B activity. Bicyclol was synthesized more efficiently from this compound in the cell culture models [24]. Another natural treatment method that has gained prominence is the use of probiotics. When used in appropriate amounts, probiotics provide health benefits to the host [25].

4. The infection process of SARS-CoV-2

Upon encountering a host cell, SARS-CoV-2 initiates the infection process by binding to the cell surface and entering the cell either through endocytosis or direct fusion with the cell membrane. When a virus infects a cell, it increases the intracellular Ca^{2} + concentration [6]. This ion is then used to activate calcium-dependent enzymes and transcription factors (Fig. 1). This activation, along with the stimulation of energy synthesis by mitochondria, promotes transcription and accumulation of the viral genome. Consequently, virions are formed and released [6,26,27].

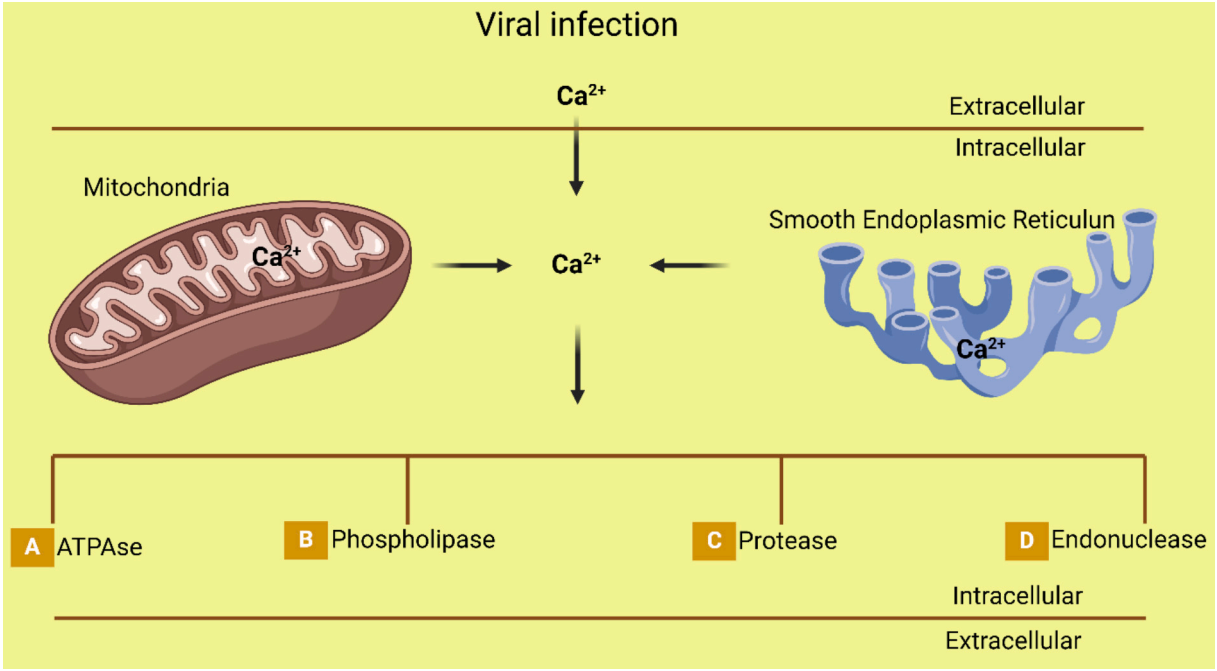

Fig. 1. Disruption of calcium homeostasis. Viral infection disrupts calcium homeostasis, leading to an increase in intracellular calcium concentration from various sources such as the external environment, mitochondria, and smooth endoplasmic reticulum. This increase activates various enzymes, including A-ATPase (reduces ATP levels), B-phospholipase (increases degradation of phospholipids, potentially leading to apoptosis), C-protease (increases protein degradation), and D-endonuclease (causes DNA damage).

5. SARS-CoV-2 exploits host Ca^{2+} signaling for efficient viral replication

The initial stages of infection involve the recognition of SARS-CoV-2 by the angiotensin-converting enzyme-2 (ACE2) receptor and fusion of its viral envelope membrane with cell membranes [28]. An in vitro study demonstrated that the S-protein's sensitivity to Ca^{2+} in a pseudovirus-liposome fusion reaction increases with higher concentrations of Ca^{2+} (ranging 100–500 μM), resulting in enhanced fusion efficiency. However, a further increase in the Ca^{2+} concentration deactivates the fusion receptor. A study on viral fusogenicity in HEK 293 T cells expressing ACE2 found that the D614G spike variant relied on Ca^{2+} for fusion [29]. Bioinformatic analysis of the S-protein revealed that SARS-CoV-2 contains a distinct RGD motif, which represents the arginine-glycine-aspartate tripeptide, in the receptor-binding domain (RBD). This motif has the potential to bind integrins found in the lung epithelium. Docking analysis suggests that the viral S-protein binds to integrins α5β1 and αvβ6, which are highly expressed in lung epithelium, through the RGD motif. Interaction between the S-protein RGD motif and a nearby sequence containing a Ca^{2+}-binding site was facilitated [30].

In a systematic study of Ca^{2+}-linked peptide molecules and lipid membranes, SARS-CoV-2-FP (a fusion peptide) penetrated the bilayer and disrupted its organization. The penetration of FP has been shown to alter the molecular organization in certain regions of the bilayer. Additionally, membrane binding of SARS-CoV-2-FP was greatly diminished in the absence of Ca^{2+} ions [28]. Lai and Freed (2021) utilized sequence alignment to identify SARS-CoV-2-FP and investigated their impact on membrane ordering. SARS-CoV-2-FP, with only three residue differences, exhibited higher membrane ordering than SARS-CoV-1-FP, primarily because of its increased hydrophobicity. The membrane order of both SARS-CoV-2 and SARS-CoV-1-FPs is Ca^{2+}-dependent; however, SARS-CoV-2 shows a stronger response in the presence of Ca^{2+}. Both FPs bind to two Ca^{2+} ions, similar to the SARS-CoV-1-FP, but the two Ca^{2+}-binding sites of SARS-CoV-2 demonstrate greater cooperativity. These results indicate that Ca^{2+} plays a crucial role in viral entry by interacting with SARS-CoV-2-FP, highlighting its significance as an important modulator. These results suggest potential therapeutic approaches that target the interaction between FP and calcium or block Ca^{2+} channels [31].

6. SARS-CoV-2 exploits host mitochondrial signaling for viral replication

Viruses depend on the molecular machinery of the host cell to survive and reproduce. Viruses manipulate cellular metabolism by regulating the structure and function of mitochondria, which are crucial centers for cellular metabolism, to evade immune responses and proliferate [32].

In response to viral infection, host cells release mitochondrial DNA and activate mitochondrial antiviral signaling proteins. This stimulates interferon production and initiates the innate immune response [32]. Gene expression analysis of SARS-CoV-2 infection revealed changes in the autophagy response, activation of innate immunity, increased reactive oxygen species (ROS) production, and decreased mitochondrial function [33].

In a recent study, the authors used protein purification mass spectrometry to identify 332 highly reliable physical interactions between the human and SARS-CoV-2 proteins. Some SARS-CoV-2 proteins interact with the mitochondrial proteins. For example, ORF9c interacts with the electron transport chain complexes and potentially regulates oxidative phosphorylation. ARF9c interacts with the outer mitochondrial membrane. The Nsp4 protein of SARS-CoV-2 has the potential to interact with the inner mitochondrial membrane [34].

The Orai1-mediated calcium signaling pathway regulates the baseline levels of type I interferon, which plays a crucial role in determining the host's resistance to SARS-CoV-2 infection. Orai1 knockout cells exhibited heightened susceptibility to SARS-CoV-2 infection, as evidenced by elevated expression of viral proteins and a substantial viral load. The knockout cells exhibited a decrease in the homeostatic cytoplasmic Ca2+ concentration and a significant deficiency in tonic interferon signaling. Transcriptome analysis revealed a decrease in various cellular defense mechanisms in Orai1 knockout cells, notably the

suppression of antiviral signaling pathways. This phenomenon is believed to stem from the diminished expression of Ca2 + −dependent transcription factors [35]. Orai1 and Orai3 have been demonstrated to act synergistically in astrocytic store-operated calcium entry (SOCE) to induce significant SOCE [36].

Transcriptomic analysis of SARS-CoV-2 infection in human-induced pluripotent stem cell-derived cardiomyocytes revealed significant changes in ion channel gene expression. Although there was an increase in the expression of certain Ca^{2+} channels, such as L-type calcium channel subunits (LTCC), transient receptor potential channel (TRP), inositol 1,4,5-triphosphate (IP_3R), and MCU, in cardiomyocytes infected with SARS-CoV-2, the levels of Ca^{2+} channel activated by Ca^{2+} release (CRAC) Orai3 and mitochondrial magnesium MRS2 channel were downregulated [37].

7. SARS-CoV-2 modulates calcium signaling in the immune system

In the immune system, calcium ions play a role in regulate the activation of immune receptors, including B-cell antigens and cytokine receptors. This leads to the release of intracellular calcium ions along with other signaling events such as the phosphorylation of cell substrates [38].

The immune system is crucial for the defense against COVID-19. However, if dysregulated and overactivated, the immune response can trigger an “cytokine storm” [39]. The uncontrolled immune response in SARS-CoV-2 is caused by the excessive secretion of pro-inflammatory cytokines, including IL-6, IL-1β, IL-10, TNF-α, and chemokines CCL2, CCL3, CCL5, CXCL9, CXCL10. Following a cytokine storm, patients with severe COVID-19 may develop immunosuppression. SARS-CoV-2 infection leads to an increase in Ca^{2+} levels in the epithelial and immune cells of patients [40].

Finding immunomodulators to rebalance the immune response against viruses is a current and future challenge for mitigating the harmful effects of viral infections [39]. B cells are activated by IL-6 and TNF-α in the bloodstream [41]. In COVID-19, the number of memory B cells is decreased [42].

8. Present-day strategies to viral disease control

Pharmacology has significantly advanced the treatment and prevention of various diseases. It was possible to observe the use of drugs during various stages of viral infection [6].

Control of certain flaviviruses, including the Japanese encephalitis virus, ZIKV, DENV, and West Nile virus, can be achieved using drugs that inhibit voltage-gated calcium channels (VGCC) (Fig. 2) during the replication phase of the virus. Examples of these drugs include manidipine, cilnidipine, and benidipine [43].

Severe fever virus with thrombocytopenia syndrome (SFTSV) is transmitted by mites, and poses a significant threat to human health [45]. Benidipine hydrochloride, a blocker of VGCC, has been shown to effectively combat this virus by inhibiting viral internalization and genome replication [46].

Drugs such as amiodarone, dronedarone, and verapamil have been shown to inhibit Ebola virus infection, similar to flaviviruses and SFTSV [47]. Fujioka et al. (2018) showed that VGCC blockers, such as diltiazem, can inhibit influenza A virus infections (H1N1 and H3N2), with are similar to flaviviruses. Interestingly, Cav1.2, a VGCC, acts as a receptor for influenza A virus. It does this by binding vira’ hemagglutinin to two sialic acids at site IV of the calcium channel. Amlodipine, nifedipine, felodipine, verapamil, and diltiazem have been shown Io inhibit SARS-CoV-2 infection by blocking the L-type calcium [44].

9. Calcium-channel blocking medication in viral infections

Several articles have been found that discussed the use of CCBs and

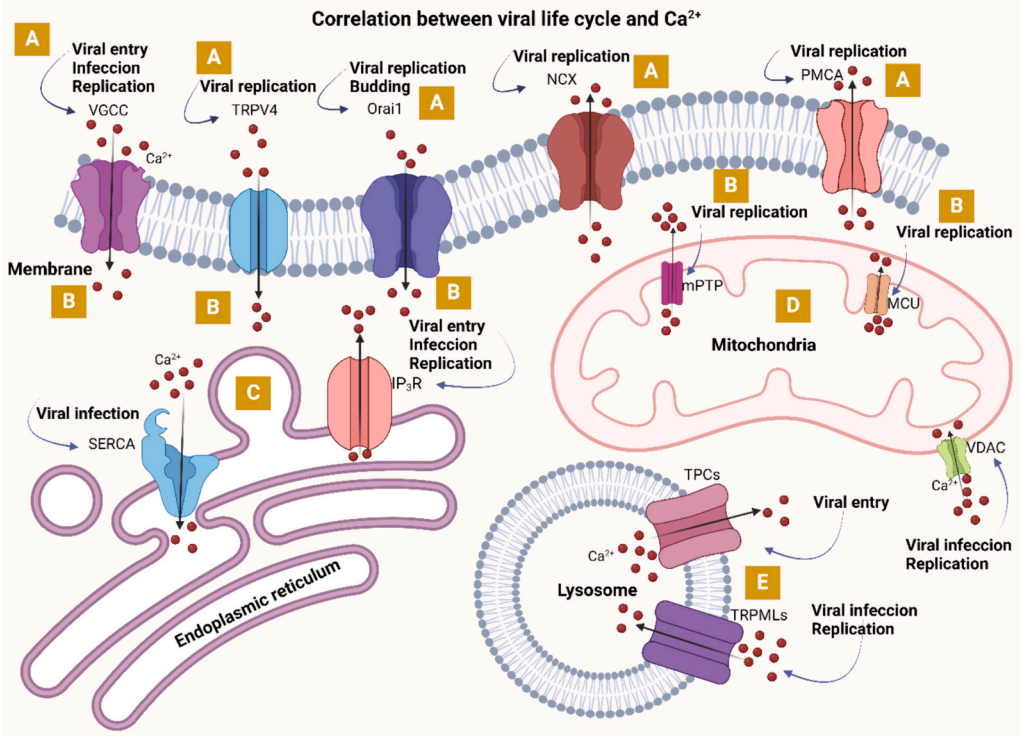

Fig. 2. Viral proteins specifically target cellular Ca2+. Several viral proteins specifically target cellular Ca^{2+} (A). Viruses can manipulate the machinery responsible for manipulating plasma membrane Ca^{2+} to increase cytosolic Ca^{2+} levels, which in turn promotes viral replication and export (B). They may target the signaling pathways of endoplasmic reticulum Ca^{2+} (C) or affect the dynamics of mitochondrial Ca^{2+} to promote viral replication and trigger apoptosis in host cells (D), ultimately facilitating viral exit. Various viruses use lysosomal Ca^{2+} efflux channels to enter host cells through the endocytic pathway I [44].

their potential to reduce virus activity in human diseases (Table 1).

10. The human microbiota and probiotics

The human and mammalian intestines consist of a diverse community of microorganisms, including bacteria, fungi, archaea, protozoa, and viruses, which together form the gastrointestinal microbiome [49,50]. The microbiome is linked to various physiological processes in the host including metabolism, energy balance, immune function, and neurobehavioral development [49]. Dysbiosis refers to an imbalance in the diversity of the gut microbiome, which can contribute to various diseases such as inflammatory bowel disease [51], gastrointestinal and metabolic conditions, and immunological and neuropsychiatric disorders [52]. Dysbiosis can occur due to antibiotic use, exposure to environmental toxins, and the consumption of processed foods. This imbalance can lead to the formation of biofilms, DNA damage from the secretion of genotoxins, and epigenetic regulation of oncogenes, potentially contributing to the development of colorectal cancer [53].

Among the members of the intestinal microbiome, numerous bacterial species can be harmful or beneficial to the host. Examples of beneficial bacterial species include *Bifidobacterium* spp. *Lactobacillus* species are commonly used as probiotics [54]. Probiotics are added to food products as supplements to help maintain the gastrointestinal balance [55]. The functions of probiotics are related to rebalancing the microbiota. For example, *Lactobacillus rhamnosus* and *L. plantarum* prevent the adhesion of enteropathogenic *Escherichia coli* [55]. Ingesting *Saccharomyces boulardii* and L. *rhamnosus* can effectively prevent or treat acute gastroenteritis, whereas L. *plantarum* can be used to prevent neonatal sepsis [56]. The regulation of host calcium homeostasis may be influenced by probiotics [57], including *Lactobacillus casei*, *L. kefiranofaciens*, *L. helveticus*, and L. *delbrueckii*. These probiotics enhance calcium transport in epithelial and intestinal cells. Additionally, *L. plantarum* increased intracellular calcium absorption in Caco-2 cells [58], a human cell line commonly used as a model of the intestinal epithelial barrier [59].

The distinction between probiotics and LBP lies in the intended use of cell lines. Probiotics are beneficial for improving patient health, particularly in patients with obesity, metabolic syndrome, and diarrhea.

Table 1
Description of articles on calcium channel blocker drugs. Brief description of articles on using calcium channel blockers to reduce viral activity in human infections.

Title	Description	Reference
Host Calcium Channels and Pumps in Viral Infections	Viruses that use calcium pumps and channels and examples of controlling their infection.	[6]
Screening of FDA-Approved Drugs for Inhibitors of Japanese Encephalitis Virus Infection	Use of Manidipine, cilnidipine and benidipine to inhibit Japanese encephalitis virus infection.	[43]
Calcium channel blockers reduce severe fever with thrombocytopenia syndrome virus (SFTSV) related fatality	The use of benidipine hydrochloride makes it difficult to internalize the virus in severe fever with thrombocytopenia syndrome.	[46]
A Sialylated Voltage-Dependent Ca^{2+} Channel Binds Hemagglutinin and Mediates Influenza A Virus Entry into Mammalian Cells	Medications based on diltiazem can prevent the binding of H1N1 and H3N2 in the calcium channel.	[48]
Ebola virus (EBOV) infection: Therapeutic strategies	The use of amiodarone, dronedarone and verapamil against Ebola virus infection.	[47]
Inhibitors of L-Type Calcium Channels Show Therapeutic Potential for Treating SARS-CoV-2 Infections by Preventing Virus Entry and Spread	Amlodipine, nifedipine, felodipine, verapamil and diltiazem demonstrated inhibitory effect on SARS-CoV-2 infection	[44]

LBP is considered medicinal and is administered to patients to prevent, treat, or cure specific diseases, such as inflammatory bowel disease (Fig. 3) [11,60].

Thus, LBP functions similar to probiotics by modulating the microbiota and affecting the immune system in the intestinal mucosa. It also plays a role in metabolite production, the activation of cellular pathways in epithelial cells, and the modulation of the nervous system [62].

NGP differ from conventional probiotics in several ways. For instance, NGP are primarily derived from commensal bacteria in the human intestine, whereas probiotics are derived from the intestine, breast milk, and fermented foods. The diversity of probiotics is often limited, with the majority being Lactobacillus and *Bifidobacterium* spp., whereas NGP have a greater diversity of genera (Table 2) [10]. Genetically modified microorganisms have also been developed and are included in this group [9].

Some NGP have shown potential as preventive and therapeutic tools during the early stages of development. Examples include *Prevotella copri* and *Christensenella minuta*, which can help regulate host insulin resistance, and *Parabacteroides goldsteinii*, *A. muciniphila*, and *Bacteroides thetaiotaomicron*, which aid in the reversal of obesity and insulin resistance [68]. *Bacteroides fragilis* is an emerging probiotic that enriches a specific subset of anti-inflammatory memory CD4 + FoxP3 T cells through direct interactions with antigen-presenting cells. This approach can effectively treat inflammation-related diseases, including abscesses, neuroinflammation, and cancer [69]. *Faecalibacterium prausnitzii* ferments glucose and produces short-chain fatty acids, including butyrate, which play crucial roles in maintaining intestinal homeostasis and integrity [68].

The STC-1 cell line was used to study the fluctuations in calcium concentration in enteroendocrine cells. A study that tested *A. muciniphila* calcium levels in the STC-1 cell line observed that the protein-enriched fraction of the conditioned medium from the mucin-free strain stimulated the release of calcium ions through ryanodine receptors (RyR), leading to increased uptake of calcium ions by mitochondria. This process results in the generation of ROS and the aggregation of αSyn, a protein found in the gut epithelium that exhibits properties similar to neurons (Fig. 4). Under mucin-free conditions, a critical metabolic state likely occurs, resulting in the production of ROS that stimulate this signaling pathway. In the presence of mucin, *A. muciniphila*, a type of bacterium known as an NGP, can break down mucin, leading to a decrease in this signaling [70].

11. Gut-lung axis

Although capable of infecting intestinal cells, SARS-CoV-2 is unable to persist due to its low pH and the presence of gastric fluid constituents such as bile and digestive enzymes [71]. However, the virus can influence the composition and function of the intestinal microbiota, leading to dysbiosis. This disruption of the gut microbial balance is linked to increased mortality rates in respiratory infections, likely due to dysregulated immune responses characterized by elevated levels of IFN-g-, IL-6, and CCL2, along with decreased regulatory T cells within both the pulmonary and gastrointestinal tracts [71,72].

Oliveira et al. (2021) suggested four mechanisms that could explain the impact of intestinal microbiota on respiratory mucosal immunity: 1) interconnection of mucosal tissues, allowing immune activation at one site to influence distant others; 2) secretion of cytokines and growth factors in the gastrointestinal tract that can affect other mucosal tissues; 3) absorption of microbial molecular patterns in extraintestinal tissues, activating pattern recognition receptors and influencing immune responses; and 4) modulation of mucosal immunity by intestinal metabolites such as short-chain fatty acids, which enhance the antiviral response in the lung. In addition to intestinal dysbiosis, patients may manifest dysbiotic microbiota in the pharynx and lungs, reinforcing the hypothesis that mucosal interconnectivity and gastrointestinal mucosal events can affect other organs.

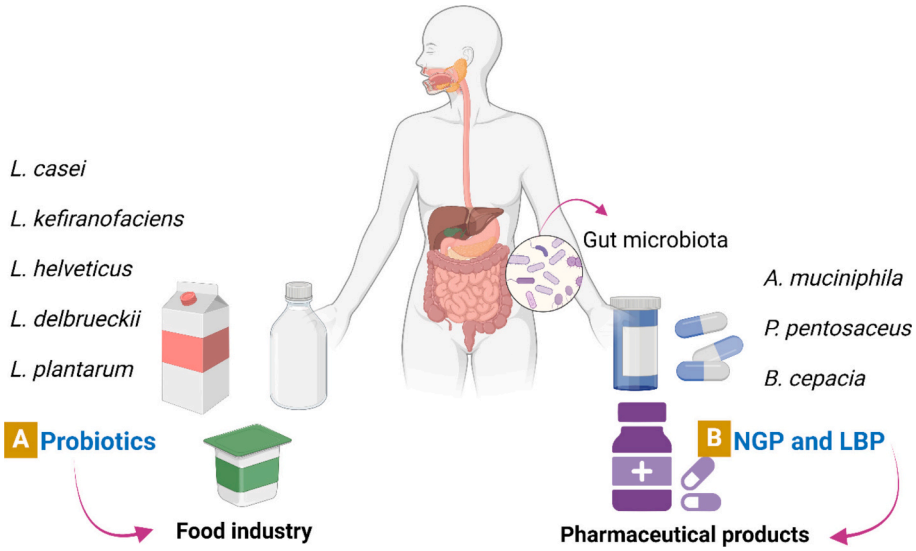

Fig. 3. Probiotics (A) are found in the food industry, and NGP and LBP (B) have potential pharmaceutical properties.

Table 2
Comparison of the studied effects of species of probiotics and next-generation probiotics.

Probiotics		Next-generation probiotics	
Genus/species	Effects studied	Genus/specie	Effects studied
Lactobacillus spp. *Bifidobacterium* spp.,	Modulation of gut microbiota and prevention of degenerative diseases [61].	*Akkermansia muciniphila*	Ameliorative effect against metabolic syndromes and auto-immune diseases in progeroid mice model [62]. Anti-obesity effect, anti-diabetic effect, and enhanced efficacy of anticancer immunotherapy in mice mode [63]. Amelioration of amyotrophic lateral sclerosis in mice [64].
Saccharomyces cerevisiae	Increase of proportion of *Bacteroidetes* and decrease of abundance of *Proteobacteria* and *Firmicutes* in gut microbiota. Ability to prevent inflammation [61].	*Pediococcus pentosaceus*	Cholesterol-lowering effect in vitro [65].
Bacillus spp.	Reduce probability of diseases and improve quality of life in aquaculture [66].	*Burkholderia cepacia*	Improves efficacy of anticancer immunotherapy of CTLA4 blockade in antibiotic-treated mice [67].

The intestinal microbiome produces various metabolites, including short chain fatty acids (SCFS). SCFS exhibits anti-inflammatory properties such as inducing apoptosis, inhibiting the cell cycle of tumor cells, and fortifying mucosal barriers against endotoxin infiltration. Ongoing studies corroborate the existence of a significant connection between the intestines and lungs. Alterations in the composition of the intestinal microbiome may be associated with increased susceptibility to respiratory diseases, changes in immune responses, and pulmonary homeostasis [73].

The intestinal microbiota can affect the gut-lung axis through segmented filamentous bacteria, which stimulate the T helper 17 cell response in the lungs, promote lung protection from *Streptococcus pneumoniae* infection, and enhance pulmonary mucosal immunity. Some probiotics can also act during viral infection through the gut-lung axis, such as SCFAs produced by *Bacteroidetes* spp. and *Clostridium* spp., which can enhance the function of influenza-specific CD8+ T cells and type I interferon (IFN) signaling in macrophages, resulting in protection against viral infection [74].

12. The role of probiotics in COVID-19 treatment

Despite the advent of vaccines that have significantly attenuated the global burden of COVID-19, research continues to develop additional strategies to combat SARS-CoV-2 and control the disease. Several studies have investigated the therapeutic potential of probiotics and natural agents capable of mitigating the effects of inflammation through diverse mechanisms [75–77].

Rather et al. (2021) showed that L. *plantarum* P88 cell-free supernatant (CFS) can eradicate most of the virus in a treatment of SARS-CoV-2 in human embryonic kidney 293 (HEK 293) infected cells, enhancing the effect as the concentration of CFS increases. The P88-CFS likely exerted a direct inhibitory effect on viral replication, possibly mediated by postbiotic substances such as PlnE and PlnF, which interfere with the activity of helicase, a crucial enzyme for viral genome replication.

One study Rather et al. (2022) showed that L. *casei* Probio 65 extract reduced SARS-CoV-2 replication by 60 % in addition to a concomitant reduction in ROS production. The extract also decreased ERK phosphorylation, suggesting that L. *casei* Probio 65 interferes with virus-cell communication during infection.

Islam et al. (2021) reported that pretreatment of Calu-3 cells, a human epithelial cell line derived from lung adenocarcinoma, with L. *plantarum* MPL16 and CRL1506 improved cell resistance during SARS-CoV-2 infection. Modulation of the immune response and reduction of inflammation are the main factors influencing the resistance enhancement of Calu-3 cells during infection. *L. plantarum* MPL16 and CRL1506 increased the production of interferon beta (IFN-β), an important antiviral protein, and also induced the expression of viral detection receptors such as TLR3 and DDX58, as well as antiviral factors like Mx1 and OAS. MPL16 and CRL1506 also significantly reduced the levels of CCL2, CXCL8, CSF3, CSF2, and CXCL10, which was important because of the high expression levels of these chemoattractants in Calu-3 cells infected with SARS-CoV-2.

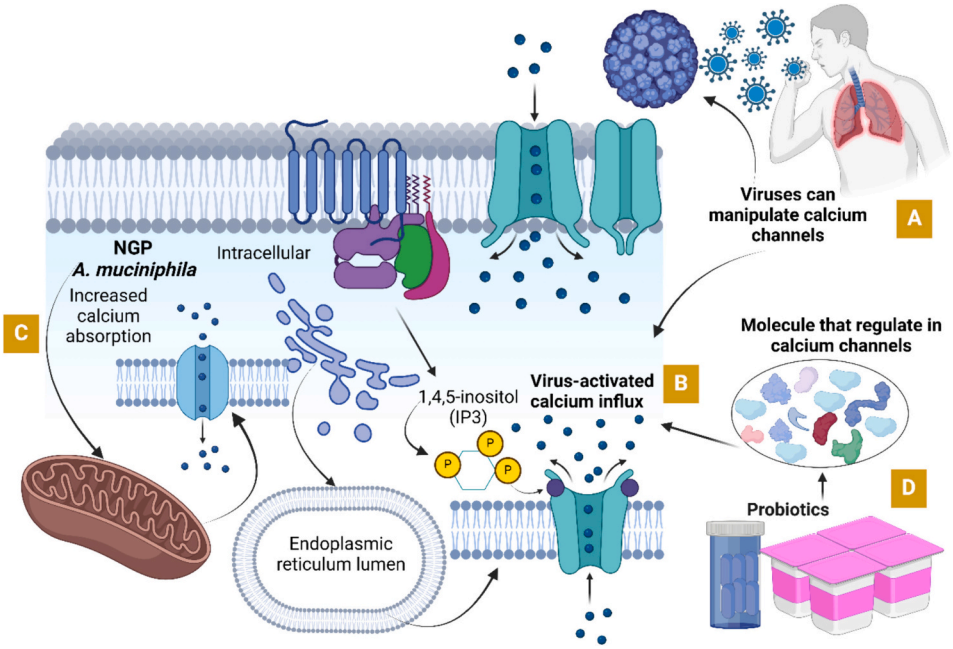

Fig. 4. Viral diseases and calcium homeostasis. Viral diseases are successful because of the ability of viruses to disrupt calcium homeostasis in cells (A). Viruses can trigger calcium influx. Conventionally, there is a higher concentration of calcium ions outside the cell. However, an excessive influx of calcium can trigger various mechanisms that can be detrimental to the cell (B). Molecules produced by NGP strains can restore calcium balance by inhibiting or reducing viral infection. *A. muciniphilla* is an example of an NGP and is linked to enhanced calcium absorption by mitochondria (C). One potential method for controlling calcium influx is through the use of probiotics. Probiotics regulate calcium channels, thereby reducing the amount of calcium that enters the cells (D).

13. Discussion

Blocking the calcium channels to interrupt viral replication may serve as an alternative method for controlling viral diseases. This approach has shown promise for flaviviruses, SFTSV, and influenza A viruses, for which certain drugs have been found to improve the disease state [43,46,48].

However, CCBs have greater affinity for specific cells, leading to unwanted side-effects. Inhibiting calcium influx can affect myocardial, vascular, and GI smooth muscle contraction, resulting in relaxation of vascular smooth muscles and subsequent vasodilation, hypotension, myocardial depression, bradycardia and atrioventricular node blockade. Additionally, these medications inhibit L-type calcium channels in pancreas islets, resulting in a reduction in insulin secretion, hyperglycemia, and reduced cardiac glucose utilization. While therapeutic doses of CCBs generally maintain tissue selectivity, toxic doses reduce this selectivity, potentially causing life-threatening bradycardia, hypotension, hyperglycemia, and renal insufficiency [21]. CCBs can cause cell damage by disrupting calcium-stimulated mitochondrial action and glucose catabolism, resulting in lactate production and ATP hydrolysis, which contributes to acidosis [78].

Another approach worth exploring is studying NCX as a means of promoting calcium efflux during viral replication, rather than relying on calcium channels that inhibit calcium influx [79]. Some drugs, including nicorandil, pinacidil, sodium nitroprussiate, sildenafil, and flecainide have been shown to perform this function in cardiac NCX [80]. This method is significant because it involves the replication of a group of viruses that use humans as hosts and can restore calcium homeostasis during virus-induced influx.

The probiotic L. *plantarum* has been associated with the improved absorption of amlodipine, a CCB. Improved absorption has been linked to reduced fatality rates in patients with COVID-19 [57,81]. However, the use of L. *plantarum* is challenging because it promotes the absorption of cellular calcium by increasing the expression of vitamin D receptors and the calcium channel TRPV6. This calcium transporter is found in the epithelial cell membranes of the intestinal tract and can lead to harmful levels of intracellular calcium [58].

Despite research involving *A. muciniphilla* and calcium, an effective solution against viral replication has not yet been found in the case of NGP. Although NGP is associated with increased calcium absorption by the mitochondria, this phenomenon can damage organelles if intensified, leading to cell death [70].

In addition, based on their usage histories, LBP still lack sufficient security measures. One important factor to consider is the antimicrobial sensitivity of the LBP strains. This is crucial for prompt treatment of unexpected reactions or allergies in patients. Another obstacle is the virulence of LBP, which refers to its potential to harm the host. Virulence factors can be identified through horizontal and vertical gene transfers [12]. However, it is important to note that any microorganism, including the intestinal microbiota, has the potential to acquire these genes.

Another important factor to consider is the potential for LBP translocations. Translocation refers to the movement of gastrointestinal microbiota components from the lamina propria to nearby lymph nodes and other organs. This can lead to infection, inflammation, and organ failure [12]. This event occurs when individuals are exposed to toxins, drugs, or pathogens that cause inflammation and disrupt the integrity of the intestinal epithelium. This disruption promotes the passage of members of the microbiota [82]. No articles directly related to the use of LBP to promote calcium homeostasis were found, likely because of the numerous obstacles in this area. With this in mind, new approaches using probiotics, NGP, and LBP can implement safe procedures to identify and remove these genes from the strains being utilized.

The use of probiotic strains for the alleviation of virus-induced diseases, with a specific focus on calcium homeostasis, presents a promising concept that warrants further investigation. Nevertheless, it is essential to consider strategies for utilizing probiotics as the interactions between these strains and target viruses may not always be advantageous to the hosts. This is similar to certain probiotics that can directly or indirectly interact with the pathogen, potentially promoting virulence [56]. Determining an appropriate measure can hinder the study of agents [25]. Most studies on the viability of these substances have been limited

to animal models, and have not been conducted in humans. Furthermore, the current level of evidence is considered moderate [9]. Allergenic molecules have been found to increase calcium influx into RBL-2H3 cells [83]. These cells have also been used to develop rapid screening methods for anti-allergic probiotics [84]. Therefore, this cell line is a valuable tool for future studies investigating the effects of NGP on calcium homeostasis.

14. Conclusions

Despite these associated risks, the use of calcium blockers to control viral infections is still being investigated. Some microorganisms, such as L. *plantarum* and *A. muciniphila*, have been found to interact with cellular calcium homeostasis. However, these interactions are associated with increased influx of calcium ions, which can lead to cell damage. Probiotics play a significant role in the food industry, whereas NGP and LBP are primarily used in the pharmaceutical industry. However, there is a lack of data on the use of these elements as agents to combat the manipulation of calcium homeostasis channels. Therefore, it is necessary to conduct further research to establish high-quality methodologies for the treatment and prevention of viral diseases.

CRediT authorship contribution statement

Hugo Massami Endo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shelon Cristina Souza Bandeca:** Writing – review & editing, Visualization. **Luiz Ricardo Olchanheski:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis. **Zelinda Schemczssen-Graeff:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Formal analysis. **Marcos Pileggi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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