

Can postbiotics show antiviral effects against Sars-CoV-2?

Podem os pós-bióticos apresentarem efeitos antivirais contra Sars-CoV-2?

Pueden los postbióticos mostrar efectos antivirales contra Sars-CoV-2?

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Abstract

Severe Acute Respiratory Syndrome of Coronavirus-2 (Sars-CoV-2) is the causative agent of the new Coronavirus Disease (COVID-19) responsible for the current pandemic that threatens global health. Although some anti-COVID-19 therapeutic agents are under investigation, there is still no evidence of antiviral action against Sars-CoV-2. Research in the literature describes the success of probiotics in the treatment of viral infections from their byproducts, known as postbiotics, such as exopolysaccharides, hydrogen peroxide, and different bacteriocins. Based on these reports, we describe the main postbiotics that present antiviral actions against different viruses with a view to suggesting their use as possible therapeutic agents for COVID-19. The revised data show promising effects for using postbiotics as efficient vehicles against various types of viruses. However, further investigation of the underlying mechanisms is required for their indication against Sars-CoV-2 and other Sars-CoV infections.

Keywords: Antiviral activity; COVID-19; New coronavirus; Probiotic.

Resumo

A Síndrome Respiratória Aguda Grave do Coronavírus-2 (Sars-CoV-2) é o agente causador da nova Doença do Coronavírus (COVID-19) responsável pela atual pandemia que ameaça a saúde global. Embora alguns agentes terapêuticos anti-COVID-19 estejam sob investigação, ainda não há evidências de ação antiviral contra Sars-CoV-2. Pesquisas na literatura descrevem o sucesso dos probióticos no tratamento das infecções virais de seus subprodutos, conhecidos como pós-bióticos, como exopolissacarídeos, peróxido de hidrogênio e diversas bacteriocinas. Com base nesses relatos, descrevemos os principais pós-bióticos que apresentam ação antiviral contra diferentes vírus, com o objetivo de sugerir seu uso como possíveis agentes terapêuticos para COVID-19. Os dados revisados mostram efeitos promissores para o uso de pós-bióticos como veículos eficientes contra vários tipos de vírus. No entanto, uma investigação mais aprofundada dos mecanismos subjacentes é necessária para sua indicação contra Sars-CoV-2 e outras infecções por Sars-CoV.

Palavras-chave: Atividade antiviral; COVID-19; Novo coronavírus; Probióticos.

Resumen

El Síndrome Respiratorio Agudo Severo por Coronavirus-2 (Sars-CoV-2) es el agente causante de la nueva Enfermedad por Coronavirus (COVID-19) responsable de la pandemia actual que amenaza la salud global. Aunque se están investigando algunos agentes terapéuticos anti-COVID-19, todavía no hay evidencia de acción antiviral contra Sars-CoV-2. Los estudios en la literatura describen el éxito de los probióticos en el tratamiento de infecciones virales causadas por sus subproductos, conocidos como posbióticos, como exopolisacáridos, peróxido de hidrógeno y varias bacteriocinas. Con base en estos informes, describimos los principales post-bióticos que presentan acción antiviral frente a diferentes virus, con el objetivo de sugerir su uso como posibles agentes terapéuticos para COVID-19. Los datos revisados muestran efectos prometedores para el uso de postbióticos como vehículos eficientes contra varios tipos de virus. Sin embargo, se necesita más investigación de los mecanismos subyacentes para su indicación contra Sars-CoV-2 y otras infecciones por Sars-CoV.

Palabras clave: Actividad antiviral; COVID-19; Nuevo coronavirus; Probióticos.

1. Introduction

Recently the world is battling a pandemic caused by a new coronavirus, called Severe Acute Respiratory Syndrome of Coronavirus-2 (Sars-CoV-2) (Velev et al., 2020). Since the first case was reported in late 2019 in the city of Wuhan - China, “Coronavirus Disease-2019” (COVID-19) has spread across the world, and according to the World Health Organization, by June 15, 2021 the number of confirmed cases had reached 176.721.173 and the number of deaths caused by COVID-19 had risen to 3.821.283 (WHO, 2021).

To date, there are no specific therapeutic agents or vaccines available for COVID-19. In fact, several drugs are under investigation, but there is still no confirmation of antiviral activity for Sars-CoV-2 (Chen et al., 2020). Therefore, there is an urgent need to search for new potent and effective anti-COVID-19 agents in order to control or prevent infection with the new coronavirus.

Various studies in the literature describe the success of using probiotics in the therapy of viral infections both *in vitro* and *in vivo* (Ermolenko et al., 2018; Sunmola et al., 2019; Abdelhamid et al., 2019). Their antiviral action can occur through such mechanisms as: (I) direct probiotic-virus interaction, (II) stimulation of the immune system and (III) production of postbiotics with antiviral action (Drider et al., 2016). Hydrogen peroxide, lactic acid, exopolysaccharides and bacteriocins are examples of postbiotics that, among other activities, also act against several viruses (Al Kassaa et al., 2014).

Thus, based on reports in the literature on the antiviral efficiency of some postbiotics, our hypothesis is that these components may play an important role in COVID-19, and therefore may aid researchers to formulate an antiviral product that can inhibit or delay Sars-CoV-2 in humans.

2. Materials and Methods

This research is documentary in nature from analyzes of articles present and registered in the scientific literature. The articles considered eligible for inclusion in this research were those that presented original studies carried out with postbiotics and evaluated for their ability to inhibit the virus, and possibly against Sars-CoV-2, published in English, Spanish or Portuguese in the years of 2011 to 2021. Non-original articles were excluded (reviews, editorials, letters and comments). Electronic databases included in PubMed, Science Direct, LILACS, Scielo, Tripdatabase and Cochrane were used.

3. COVID-19 and Treatment Perspectives

Effective drugs or vaccines that can be used to prevent or mitigate the effects of Covid are still not available. Thus, some countries have adopted non-pharmacological, (NPI)-based intervention strategies to contain the virus and the transmission of the disease, such as by enforcing social distancing, self-isolation, quarantine and even lockdown (Di Grezia et

al., 2020). However, different classes of molecules have been reported, in the literature, regarding their potential against SARS-CoV-2.

3.1 Postbiotics: What do we Know About their Virucidal Potential?

Although the concept of probiotics indicates that microorganisms must be alive active to provide benefits to hosts, evidence suggests that microbial viability is not necessary to achieve such effects. Some proposals indicate that probiotic substances, known as postbiotics, can also benefit the health of the host (De Almada et al., 2017; Martín et al., 2019). These products can be short-chain fatty acids (SCFAs), microbial fractions, proteins or functional enzymes, secreted or extracellular polysaccharides (EPS) and teichoic and lipoteichoic acids (Markowiak et al., 2019), which have different actions, among which are anti-inflammatory, immunomodulatory, antioxidant and antimicrobial activity (Vallejo et al., 2020). However, the evidence on mechanisms of action by which these products exert their specific effects in certain systems or diseases has still not been completely elucidated (Barros et al., 2020; De Almada et al., 2016).

3.2 Exopolysaccharides

Exopolysaccharides (EPS) from probiotic lactic acid bacteria (LAB) are natural biopolymers composed of sugars and have been used for various applications, mainly for biological activities *in vitro* as well as *in vivo* (Freitas et al., 2011; Badel et al., 2011). Researchers have reported that this biological high-molecular long-chain polysaccharide can be a potential inhibitor of viral infection, especially in the systemic and respiratory. There follow examples of studies in which the EPS of probiotic bacteria has been used as agents against some known virus diseases.

Metabolites produced by *Lactobacillus plantarium* proved to be effective against Transmissible Gastroenteritis Virus (TGVE) infection, a member of the Coronaviridae family (Yang et al., 2017). The authors observed a reduction of TGEV proliferation up to 78% by administering 1/4 dilution of a metabolic product of *L. plantarium*, and they later found that the major component of the metabolic product was EPS. Biliavska et al., (2019) reported that EPS produced by *Lactobacillus spp.* strain was capable of releasing the human adenovirus type 5 (HAdV-5) from cells after virus adsorption. Kim et al., (2018) investigated the effects of EPS from *L. plantarium* against rotavirus *in vitro* and in infected neonatal mice. They concluded that EPS displayed a high rate of adhesion and thus interfered with the rotaviral attachment to the cells *in vitro*. They reported that EPS decreased the rotavirus replication in the intestine of mice and reduced the symptoms after rotavirus infection: limited epithelial lesion, reduced episodes of diarrhea, and shortened the time to recovery of suckling mice. Nagai et al. (2011) found that mice infected by influenza virus and treated with yogurt and EPS of *L. delbrueckii* and *L. bulgaricus* showed a decrease of influenza virus titer. Compared to groups treated with water, there was a significant increase of anti-influenza virus antibodies (IgA, IgG) in the bronchoalveolar lavage fluid at 4 days post-infection NK cell activity of splenocytes in both groups. According to Vivier et al. (2008) NK cells are important lymphocytes that play a crucial role in the defense against various virus infections. Thus, the authors concluded that yogurt fermented with EPS produced by *L. bulgaricus* can act on the immune system and protect against influenza virus infection.

Based on several studies, EPS therapy may be a candidate for reducing the severity of the COVID-19 infection or even for inhibiting the entrance of the virus into cells. According to Biliavska et al. (2019), bacterial exopolysaccharides show significant antiviral activity due to the degradation of the viral particles, a decrease in the titer of viruses, the blocking of viral DNA replication, and the release of the infectious virus particle. However, the antiviral mechanism of EPS has not been studied sufficiently. It is known that stimulation of the immune system occurs. *Lactobacillus* and its exopolysaccharides can stimulate the synthesis and accumulation of interleukin 12 to enhance the activity of natural killer cells and the synthesis of IgA. By activating the immune response and producing IgA, it was possible to decrease the influenza virus infection (Jung et

al., 2017). As shown, some probiotic strains such as from *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* with their EPS were indicated to show stronger therapeutic effect against several types of virus: adenovirus type 5, rotavirus, gastroenteritis corona virus and influenza virus (Biliavska et al., 2019; Kim et al., 2018; Yang et al., 2017).

3.3 Hydrogen peroxide

Hydrogen peroxide (H₂O₂) is an important substance in the metabolism of living organisms, produced by several bacteria including probiotics (Forman et al., 2008). According to Valko et al. (2007) oxidizing agents such as H₂O₂, represent a key element of the innate mammalian immune system and function in endosomal compartments to inactivate intracellular pathogens.

H₂O₂ plays an important role as a defense mechanism to prevent contamination by opportunistic microorganisms. The antiviral effects of H₂O₂ have been studied for a long time. Klebanoff et al. (1991) reported that *Lactobacillus* found in the vaginal environment can produce H₂O₂ as a natural microbicide that can be toxic to a number of viruses, including HIV and hsv-2. The authors observed that in vitro tests revealed the amount of H₂O₂ generated by 10⁷ organisms was sufficient to inactivate HIV. Studies also demonstrated that H₂O₂ can be a means for producing viral vaccines. Amanna et al. (2012) developed a new vaccine based on H₂O₂ to inactivate viruses. They observed that neutralizing antibody and CD8+ T cell responses that were induced by H₂O₂ had an effective antiviral function. They concluded that H₂O₂ conferred protection against a range of viral pathogens and improved antigenicity and immunogenicity when compared to other standard approaches used for virus inactivation (formaldehyde and β-propiolactone).

Due to its antiviral activity and favorable safety profile, H₂O₂ from probiotic bacteria can be considered as antiviral candidates to be further evaluated against Sars-Cov-2 infection. To date, studies have only reported the effect of H₂O₂ as a virucidal agent for material and surface disinfection. For example, Ibáñez-Cervantes et al. (2020) reported that H₂O₂ was efficient at disinfecting N95 masks contaminated by Sars-Cov-2 and Kampf et al. (2020) found that 0.5% of H₂O₂ solution is effective at inactivating human coronaviruses such as Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) on inanimate surfaces Kampf et al. (2020). However, the use of H₂O₂ in the nose, mouth and throat in a specific concentration is clearly indicated to enhance those local innate responses to viral infection against coronavirus (Caruso et al., 2020).

3.4 Bacteriocins

Bacteriocins are ribosomally synthesized peptides by several lactic acid bacteria that have a bactericidal or bacteriostatic effect against related or unrelated bacteria (Al Kassaa et al., 2016) and potential for inhibition against several viruses. However, the repertoire of bacteriocins that have antiviral activity includes some that have been reported in several studies but the mechanisms of these bacteriocins are still little known (Aspri et al., 2016).

Bacteriocins synthesized by *Enterococcus durans* isolated from goat milk were evaluated for antiviral activity against herpes simplex virus 1 (HSV-1) and poliovirus (PV-1) in Vero cells (Cavicchioli et al., 2017). The authors observed that the bacteriocin GEn17 had the best percentage (71.6%) of HSV-1 inhibition, with a 50% inhibitory concentration (IC₅₀) of 24 µg / mL and a selectivity index (SI) of 17.8, while GEn09 showed the best antiviral performance against PV-1, reaching 92.2% inhibition, with IC₅₀ of 22.2 µg / mL and SI of 25.8. These results indicate that the bacteriocins produced by *E. durans* proved to be effective in inhibiting this virus.

On the other hand, pediocin-like bacteriocin ST5Ha synthesized by *E. faecium* ST5Ha was active against HSV-1 virus with selectivity index 173 and IC₅₀ of 50 µg / mL in Vero cells (Todorov et al., 2010). A non-cytotoxic bacteriocin produced by

Lactobacillus delbrueckii subsp. bulgaricus 1043 demonstrated virucidal activity against H7N7 and H7N1 influenza virus, with IC₅₀ of 5.6 ng/ mL and 4.2 ng/ mL, respectively (Serkedjieva et al., 2000).

HSV-1 and HSV-2 show susceptibility to the peptide ST4V synthesized by *E. mundtii* ST4V in Vero cells of the kidney of the African green monkey in a dose-dependent manner with a 99.9% inhibition percentage of the two viruses at an IC₅₀ value of 400 µg/ mL (Todorov et al., 2005).

The results reported by Wachsman et al. (2003) also indicate that enterocin CRL35 by *E. faecium* showed antiviral activity with an IC₅₀ value of 15 µg/ mL against HSV-2 in Vero cells. They also point out that this virucidal activity may be due to inhibition of late protein synthesis such as extracellular D-glycoprotein responsible for viral assembly, since 25 µg/ mL of CRL35 was able to reduce 65% of the production of this protein.

The anti-influenza activity of enterocin-B by *E. faecium* L3 was also assessed and its IC₅₀ of 5 µg/ mL caused 100% inhibition of H3N2 virus reproduction and 33% inhibition of H1N1 virus in the MDCK cell line. Likewise, when this strain of *E. faecium* L3, at a dose of 1 x 10⁷ CFU, was evaluated for the health and survival of mice affected by H1N1 influenza viral infection, 20% of the mice in the experimental group recovered in contrast with the group without the probiotic all of which died (Ermolenko et al., 2018).

Recently, metabolites of *Lactobacillus plantarum* from the plantaricin category were selected to design an antiviral computational product to slow, inhibit or kill the new Sars-CoV-2. Plantaricin was able to bind tightly by blocking RdRp (RNA-dependent RNA polymerase), the residual binding domain (RBD) of spike protein S and ACE2 (angiotensin-converting enzyme 2) with binding energies -14.7, -11.1 and -12.7, respectively (Anwar et al., 2020).

4. Final Considerations

We have reported on postbiotics with antiviral effects found in the literature. Several research groups have reported the promising effects of postbiotics as an efficient vehicle against several types of viruses. However, further investigation of the underlying mechanisms is required. As no therapeutic agents for COVID-19 have as yet been approved, there is an urgent need to investigate bioproducts such as postbiotics and to test them on their own or combined with probiotic strains against the current COVID-19 and other CoV infections.

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