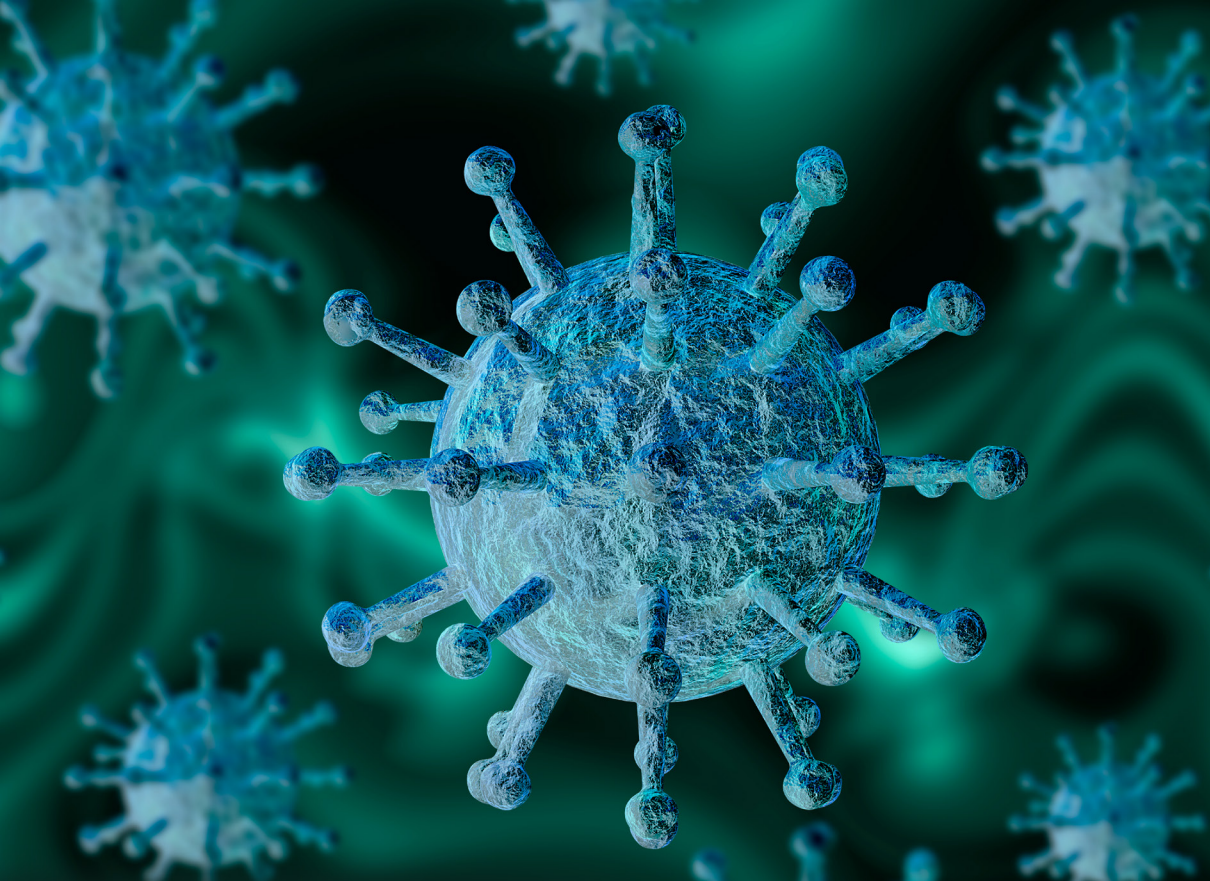

Larissa Maranhão Dias
(Organizadora)

Microbiologia:

Geração de conhecimento e caráter multidisciplinar

2

Atena Editora
Ano 2022

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APRESENTAÇÃO

Desde a criação do microscópio e com ele a descoberta do mundo microscópico os microrganismos passaram a ser de interesse comum a diversas áreas; inicialmente na saúde e conforme suas descobertas esta temática ramificou-se para outros campos, como as ciências biológicas e nas áreas de ensino. Atualmente, a Microbiologia é um assunto em crescimento exponencial.

Assim, de forma colaborativa e integrada o volume "Microbiologia: Geração de conhecimento e caráter multidisciplinar 2" apresentada nesta edição reúne estudos desenvolvidos em instituições de ensino brasileiras que contribuem na grande área da Microbiologia através de pesquisas de cunho experimental e de caráter bibliográfico.

Esta obra tem início com o uso da metodologia de sala de aula invertida no ensino de graduação para o componente curricular de Microbiologia de Alimentos, realizado durante a pandemia de Covid-19. Esta análise teve um rendimento positivo com a metodologia utilizada, contribuindo de forma significativa com a aprendizagem dos discentes.

Após, é apresentado uma pesquisa que relata a pressão seletiva sob os microrganismos em função da pandemia do Covid-19. Neste artigo, apresenta de que forma o uso inadequado de antimicrobianos de amplo espectro contribui na propagação de bactérias resistentes aos principais antibióticos usados em âmbito hospitalar. Ainda relacionado a área da bacteriologia, a terceira seção deste volume conta com um experimento que envolveu análise de amostras biológicas oriundas de profissionais da saúde, de um hospital público, contaminados por *Staphylococcus aureus* com perfil de resistência a antimicrobianos. Esta análise traz a importância do emprego correto dos EPI'S e hábitos de higienização.

Além disso, essa publicação conta com três trabalhos que abordam a área da Micologia, presentes no quarto, quinto e sexto capítulos, respectivamente. O quarto estudo prop***õem*** uma alternativa sustentável para uso de resíduos quitinosos oriundos por indústrias de frutos do mar através de quitinases fúngicas por processos biotecnológicos. A seção seguinte relata sobre infecções da mucosa oral causadas pelo fungo oportunista *Candida* e uma alternativa de mitigar este cenário através da utilização de filmes oroadesivos associados com produtos naturais. Por fim, o último capítulo discute sobre o monitoramento da qualidade do ar devido a presença de esporos de fungos anemófilos em suspensão, que podem desencadear infecções sistêmicas graves em indivíduos imunocomprometidos.

Reconhecemos o potencial dessa obra em primeiro lugar pela qualidade dos trabalhos aqui apresentados, e em segundo pelo campo em potencial, corroborando para futuras novas discussões na área microbiológica.

Assim desejo a todos uma ótima leitura!

Larissa Maranhão Dias

SUMÁRIO

SUMÁRIO

FUNGAL CHITINASES: CULTIVATION, PRODUCTION AND BIOTECHNOLOGICAL APPLICATION

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ABSTRACT: Chitinases are enzymes of the chitinolytic complex, responsible for hydrolyzing the β-1,4 linkages of *N*-acetylglucosamine present in the chitin polymer, a substrate that is found mainly in the exoskeletons of crustaceans, insects and the cell wall of fungi. Due to a large amount of chitinous waste that are generated mainly by the seafood industry worldwide, there is a need and importance to search for methods for using and degrading these compounds. Considering the imminent need to solve this gap due to the environmental consequences generated by the incorrect disposal of these waste, the use of enzymatic technology based on fungal chitinases comes as an alternative strategy to contribute to more sustainable methodologies. Thus, this study aimed to address the properties of chitinases and cultivation methodologies for their production from fungi described in the scientific literature, as well as to investigate the latest advances in practical applications of these enzymes.

KEYWORDS: Chitin, chitinase, chitinolytic waste.

QUITINASES FÚNGICAS: CULTIVO, PRODUÇÃO E APLICAÇÃO BIOTECNOLÓGICA

RESUMO: As quitinases são enzimas do complexo quitinolítico, responsáveis em hidrolisar as ligações β 1,4 de *N*-acetilglicosamina presente no polímero da quitina, que é encontrada principalmente em exoesqueletos de crustáceos, insetos e parede celular de fungos. Devido a elevada quantidade de resíduos quitinosos gerados principalmente pela indústria de frutos do

mar no mundo todo, ressalta a necessidade e importância pela busca de métodos para aproveitamento e degradação desses compostos. Considerando a necessidade eminente de solucionar essa lacuna devido as consequências ambientais geradas pelo descarte incorreto desses resíduos, o uso de tecnologia enzimática a base de quitinases fúngicas vem de encontro como estratégias alternativas para contribuir com metodologias mais sustentáveis. Assim, esse estudo teve como objetivo abordar sobre as propriedades das quitinases e metodologias de cultivos estratégicos de produção de quitinases de fungos descritos na literatura científica, bem como averiguar sobre os últimos avanços de aplicações práticas dessas enzimas.
PALAVRAS-CHAVE: Quitina, quitinase, resíduos quitinolíticos.

1 | INTRODUCTION

Chitin is a biopolymer made up of *N*-acetylglucosamine residues linked by β-1,4 bonds, abundantly present in nature and found in the exoskeleton of crustaceans, insects, the cell wall of fungi and some algae (EL KNIDRI et al., 2019; VALLEJO-DOMÍNGUEZ et al., 2021).

Due to its characteristics such as non-toxicity, biocompatibility and biodegradability, the biotechnology industry has used this compound in different areas, including biomedical, pharmaceutical, food, cosmetics and agriculture (TOLESA; GUPTA; LEE, 2019). In addition, chitin is also renewable and sustainable, which makes its use advantageous for various sectors (HUANG et al., 2018).

In 2018, 9.4 million tons of crustaceans were produced worldwide; 50% to 70% of the volume processed by industries is potential raw material from which most of the chitin used in the industry has been extracted (FAO, 2020; HUANG et al., 2018; MAO et al., 2017; MOHAN et al., 2021).

However, conventional chitin extraction methods use chemical compounds such as sodium hydroxide (NaOH) to remove proteins and hydrochloric acid (HCl) to remove minerals, and are procedures that make the process more expensive, in addition to generating large amounts of contaminating compounds (HU et al., 2020; SANTOS et al., 2020).

Thus, the use of biological methods, such as the use of enzymes, appears to be a good option for the improvement of existing methodologies. Such methods use the specificities of each enzyme, as well as milder reaction conditions, such as temperatures ranging from 25 to 60 °C, to obtain chitin, where proteases, for example, remove the proteins present in the residues (SANTOS et al., 2020).

In addition, the products obtained from the enzymatic hydrolysis of chitin, called chitooligosaccharides (COS), have different attractive characteristics for the industry, including their antibacterial, antifungal, antioxidant, anti-inflammatory and immunostimulatory activity (JAFARI et al., 2020).

Biotechnology industries already use a wide variety of enzymes; fungi, especially filamentous fungi, play a prominent role in the production of these molecules (POMMER et

al., 2021).

These microorganisms naturally secrete chitinases which play several and important roles for their survival, acting in nutrition, morphogenesis, mycoparasitism, remodeling and cell growth, and even in apoptosis (HALDER; PAL; MONDAL, 2019).

In this context, this study aimed to address the characteristics and properties of chitinases produced by fungi, as wells as the types of cultivation used to obtain them and practical biotechnological applications of the enzymes.

2 | CHITINASES

Among the various hydrolytic enzymes produced by fungi, there are chitinases, which were discovered in 1921 by Folpmers. These enzymes are responsible for hydrolyzing the β-1,4 glycosidic linkages of chitin, releasing oligosaccharides of *N*-acetylglucosamine and, according to the site of cleavage, they can be divided into endochitinases and exochitinases (CHEN; JIANG; YANG, 2020; DU et al., 2021; FLEURI; SATO, 2008).

Endochitinases (EC 3.2.1.14) randomly hydrolyze the internal linkages of chitin, generating soluble compounds with lower molecular weight, such as chitotrioses and chitotetraoses (KARTHIK et al., 2014; NAGPURE; CHOUDHARY; GUPTA, 2014).

On the other hand, exochitinases are composed of three enzymes that carry out hydrolysis at specific points of chitin, exochitinase (EC 3.2.1.200) responsible for cleavage at the non-reducing end, exochitinase (EC 3.2.1.201) that performs cleavage at the reducing end and produces diacetylchitobiose, and β-*N*-acetylglucosaminidases (EC 3.2.1.52) responsible for the release of *N*-acetylglucosamine monomers (Fig. 1) (BERINI et al., 2018; DWYER et al., 2021).

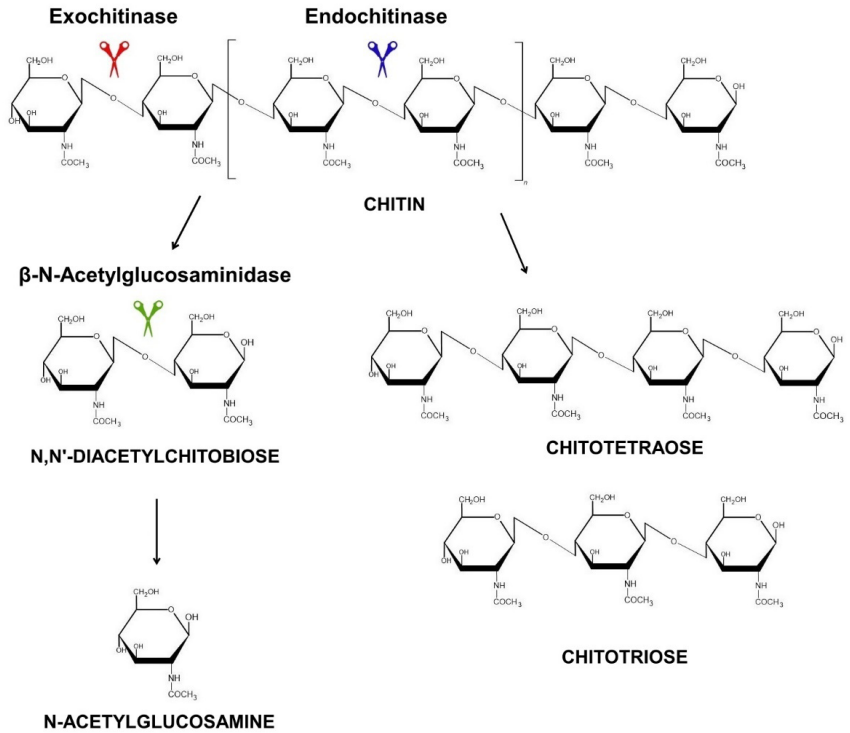

Figure 1 - Mode of action of chitinases. The scissors indicate the site of cleavage by the enzymes in the chitin structure. (✂) exochitinase; (✂) endochitinases; (✂) β-*N*-acetylglucosaminidase (Source: adapted from Nagpure, Choudhary & Gupta, 2014).

Most fungal chitinases belong to the GH18 family of glycosyl hydrolases composed of chitinases that are also produced by bacteria and viruses. Their structure is formed by five regions: a catalytic domain, N-terminal signal peptide region, chitin-binding domain, serine-threonine-rich region and C-terminal extension, the last three absent in most fungal chitinases (DENG et al., 2019; FUNKHOUSER; ARONSON, 2007; HAMID et al., 2013).

Furthermore, some fungal β-*N*-acetylglucosaminidases have already been described as belonging to the GH3 (YANG et al., 2014) and GH20 families (CHEN et al., 2015; QU et al., 2021).

These enzymes can be divided into three groups (A, B and C), based on the amino acid sequence of the GH18 module; they differ in the substrate-binding site, in their catalytic action and in the chitin-binding region (KARTHIK et al., 2014; KHAN et al., 2015).

Subgroup A chitinases have a catalytic site, but not a chitin-binding region, with a molecular mass ranging from 40 to 50 kDa. Subgroup B has chitinases of different sizes, with a molecular mass ranging between 30 and 90 kDa, whose smaller proteins contain

the chitin-binding region, while the larger ones are bound to the plasma membrane. And the C subgroup is relatively new and comprises chitinases with sizes ranging between 140 and 170 kDa and which have the chitin-binding region (KARTHIK et al., 2014; KHAN et al., 2015).

Subgroup C chitinases have their GH18 module located inside the protein and have the N-terminus composed of a carbohydrate-binding module (CBM18) and two modules of LisM, a protein domain involved in binding to polymers containing *N*-acetylglucosamine (GRUBER et al., 2011; PEREIRA et al., 2019).

However, fungal chitinases are still not well classified; they have been identified based on their similarity to chitinases from the GH18 family of plants and bacteria (HAMID et al., 2013).

3 I PRODUCTION OF FUNGAL CHITINASES

Different microorganisms have already been identified as producers of chitinases, but fungi are the main ones cited in the literature. In addition, chitinases can be found in insects, viruses, plants and vertebrates (LE; YANG, 2019).

Although the production of chitinases by yeasts such as *Saccharomyces cerevisiae* (ABDEL-KAREEM; RASMEY; ZOHRI, 2019) and *Candida albicans* (SELVAGGINI et al., 2004) has already been reported, filamentous fungi are those most used in the production of these enzymes; some of the genera used are *Trichoderma* (BALDONI et al., 2020; LOC et al., 2020), *Penicillium* (XIE et al., 2021), *Aspergillus* (ABDEL WAHAB et al., 2022), *Metarhizium* (DOS REIS et al., 2018), *Beauveria* (SCHMALTZ et al., 2021) and *Thermothelomyces* (POMMER et al., 2021).

A wide variety of genes encoding chitinases are found in fungal genomes. Filamentous fungi, in general, have a greater number of genes for chitinases, such as the fungi *Trichoderma virens* with 36 genes, *Beauveria bassiana* with 20 genes and *Trichoderma reesei* and *Aspergillus fumigatus* which each have 18 genes (ALCAZAR-FUOLI et al., 2011; JUNGES et al., 2014; LANGNER; GÖHRE, 2016; XIAO et al., 2012).

In contrast, yeasts have a lower number of chitinolytic genes, such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, which have two genes and one gene, respectively (KARLSSON; STENLID, 2008).

Thus, chitinases act in different biological functions important for fungi, such as nutrition and in the degradation of chitinolytic compounds to obtain simple carbohydrates (RONCERO; VÁZQUEZ DE ALDANA, 2020), such as chitinases from the fungus *T. atroviride*, whose *tac2*, *tac3*, *tac6* and *tac7* genes are expressed in the presence of colloidal chitin (GRUBER et al., 2011) and *T. virens* which has 15 chitinolytic genes, *tvc2*, *tvc3*, *tvc4*, *tvc5*, *tvc6*, *tvc7* and *tvc10* being expressed in the presence of colloidal chitin and *tvc4*, *tvc7* and *tvc10* expressed in the presence of crude chitin (GRUBER; KUBICEK; SEIDL-

SEIBOTH, 2011).

Chitinases are also expressed during mycoparasitism, when fungi compete in the environment, and they affect the interaction between fungal cells, exemplified by the fungus *T. virens* in which the chitinase genes *tvc2*, *tvc3*, *tvc4* and *tvc10* are expressed when cultured with both *Botrytis cinerea* and *Rhizoctonia solani* fungi. However, when inoculated alone, this induction does not occur, indicating that this action occurs due to the presence of other live fungal cells (GRUBER; KUBICEK; SEIDL-SEIBOTH, 2011).

The fungus *Trichoderma harzianum* also expresses chitinolytic genes during contact with the fungus *Ganoderma boninense*, indicating that chitinases are present during the mycoparasitism process (NAHER et al., 2018).

Furthermore, chitinases are expressed during autolysis, a process in which fungal cells rupture and is important for the release of nutrients present there; it occurs in old cultures and is affected, for example, by colony aging, lack of nutrients and apoptosis (GRUBER; SEIDL-SEIBOTH, 2012). The fungus *Hypocrea atroviridis* expresses *chi18-2*, *chi18-3* and *chi18-5* genes during carbon or nitrogen starvation (SEIDL et al., 2005). The fungus *T. virens* expresses *tvc3*, *tvc4*, *tvc5*, *tvc6*, *tvc7*, *tvc9* and *tvc10* genes during a lack of nutrients (GRUBER; KUBICEK; SEIDL-SEIBOTH, 2011).

Chitinases are also present in morphogenesis, acting in cell growth and expansion of these fungi, as reported for the fungus *Trichoderma asperelloides*; its 17 chitinase genes are expressed during germination of the microorganism's conidia (GORTIKOV et al., 2022). Furthermore, chitinase genes from the fungus *T. virens* are abundantly expressed under culture conditions (without an inducing carbon source), but gene expression may vary during fungal growth according to the cultivation mode, and in different locations on the hyphae (GRUBER; KUBICEK; SEIDL-SEIBOTH, 2011).

4 | CULTURE CONDITIONS TO OBTAIN CHITINASES FROM FUNGI

During the process of obtaining chitinases, some parameters directly affect enzyme production, including the carbon source and the type of cultivation (STOYKOV; PAVLOV; KRASTANOV, 2015).

Most chitinases are extracellular, their production could be induced by chitin present in the external environment, and are secreted by microorganisms that consume this chitin as a source of nitrogen and carbon(DAS; ROY; SEN, 2016; STOYKOV; PAVLOV; KRASTANOV, 2015).

Thus, the main cultivation condition for obtaining chitinase has been submerged fermentation (SmF) (Tab. 1), due to its practicality to control the process and the ease of recovering the enzymes produced (KARTHIK; BINOD; PANDEY, 2017). However, this technique makes it possible to obtain more diluted products when compared to solid-state cultivation (KARTHIK et al., 2014; STOYKOV; PAVLOV; KRASTANOV, 2015).

Fungi	Cultivation Condition	Carbon Sources	Reference
Alternaria alternata	SmF	Shrimp shell	(GHANEM; AL-FASSI; FARSI, 2011)
***Aspergillus flavus* CFR 10**	SSF	Wheat bran + α-chitin powder	(SURESH; ANIL KUMAR, 2012)
***Aspergillus niger* LOCK 62**	SmF	Colloidal chitin	(BRZEZINSKA; JANKIEWICZ, 2012)
Aspergillus terreus	SmF	Fish scale	(GHANEM; AL-GARNI; AL-MAKISHAH, 2010)
***Beauveria bassiana* IBCB 66**	SmF	Rice and soy bran	(SCHMALTZ et al., 2021)
***Fusarium oxysporum* CFR 8**	SSF	Wheat bran + α-chitin powder	(SURESH; ANIL KUMAR, 2012)
***Myceliophthora thermophila* C1**	SmF	Glucose	(KROLICKA et al., 2018)
***Penicillium monoverticillium* CFR 2**	SSF	Wheat bran + α-chitin powder	(SURESH; ANIL KUMAR, 2012)
Penicillium oxalicum k10	SSF	Chitin powder	(XIE et al., 2021)
Thermothelomyces heterothallicus PA2S4T	SmF	Orange peel flour	(POMMER et al., 2021)
Trichoderma asperellum PQ34	SmF	Colloidal chitin	(LOC et al., 2020)
Trichoderma koningiopsis UFSMQ40	SSF	Wheat bran + chitin powder	(BALDONI et al., 2020)
***Trichoderma viride* AUMC 13021**	SmF	Colloidal chitin	(ABU-TAHON; ISAAC, 2020)

SmF: submerged fermentation; SSF: solid state fermentation.

Table 1 - Cultivation conditions and carbon sources used for fungal chitinase production

5 | BIOTECHNOLOGICAL APPLICATIONS OF FUNGAL CHITINASES

Chitinases are used in a variety of industries, including food and medicine as well as agriculture (BHAGWAT et al., 2021). These enzymes are important in the areas of control of pathogenic organisms such as fungi and insects, in the degradation of chitinous residues of crustaceans (BARGHINI et al., 2013) and in the isolation of protoplasts from fungi and yeasts (AKEED; ATRASH; NAFFAA, 2020).

In the food industry, their application can increase food conservation, eliminating spores that may interfere with the shelf life of a product (LE; YANG, 2019), as demonstrated by the increase in the shelf life of cherry tomatoes coated with alginate biofilm containing chitinase that inhibits the proliferation of *Fusarium oxysporum* (WU et al., 2022).

For agriculture, the use of methodologies that use chitinolytic enzymes to control pathogenic insects and fungi is advantageous since treatments with traditional fungicides and pesticides, in addition to the high cost of application, are harmful to other organisms and the environment (NAGPURE; CHOUDHARY; GUPTA, 2014). For example, the chitinase

produced by *Trichoderma asperellum* PQ34 exhibits antifungal activity and inhibits growth of the phytopathogenic fungi *Colletotrichum* sp. and *Sclerotium rolfsii*, responsible for damage in mango and pepper crops and peanuts, respectively (LOC et al., 2020).

In addition to their intrinsic applications, the products generated through the hydrolysis of chitins by chitinases have great biotechnological potential, since COS have anti-inflammatory, antioxidant, antitumor, antimicrobial and tissue regenerative properties (FILHO et al., 2020; LIAQAT; ELTEM, 2018).

The antioxidant activity of COS was observed by inhibiting the formation of carbonyl groups in membrane proteins of RAW264.7 cells that were exposed to hydroxyl radicals; the oligosaccharides prevented more than 60% of the oxidation of membrane proteins at a concentration of 100 g/mL (NGO et al., 2011).

In addition, the cytotoxic activity of COS with a degree of polymerization (DP) from 3 to 7 was observed in breast cancer cell lines; the COS molecules inhibited the growth of these cells, as well as inducing their apoptosis (MALLAKUNTLA et al., 2021). Also, COS have been reported to stimulate tumor necrosis factor-α (TNF-α) in cells, thus demonstrating anti-inflammatory activity (SANTOS-MORIANO et al., 2018).

6 | CONCLUDING REMARKS

Most of the studies described in recent years in the scientific literature report a concern to contribute to the reduction of environmental pollution due to the accumulation and generation of chitinolytic residues produced annually in the world.

There is a strong tendency to look for enzymatic technologies as they are milder and more environmentally sustainable compared to chemical methodologies. Many studies report the use of chitin-based residues as inducing carbon sources or prospecting agents for chitinases by several species of fungi under submerged fermentation.

As a result, studies have intensified to understand the mode of action of chitinases and improve or optimize their production by fungi, as well to investigate their applications in various biotechnological sectors, with the purpose of finding more effective practical processes, since most of the results described have been at the academic level and laboratory scale, although there are products based on chitinases used mainly in the agricultural area, in the biological control of phytopathogens and also in the food area as biopreservatives.

Furthermore, the use of chitooligosaccharides (COS), which are chitin degradation products, has also been growing and gaining prominence in the medical and pharmaceutical sectors, due to their antitumor and antioxidant properties. These COS also appear to have the potential for employment in the treatment of diseases such as cancer.

Despite the advances in research in recent years, studies are still needed for a better understanding of the gene expression of these chitinases, aiming to improve their

acquisition and industrial application.

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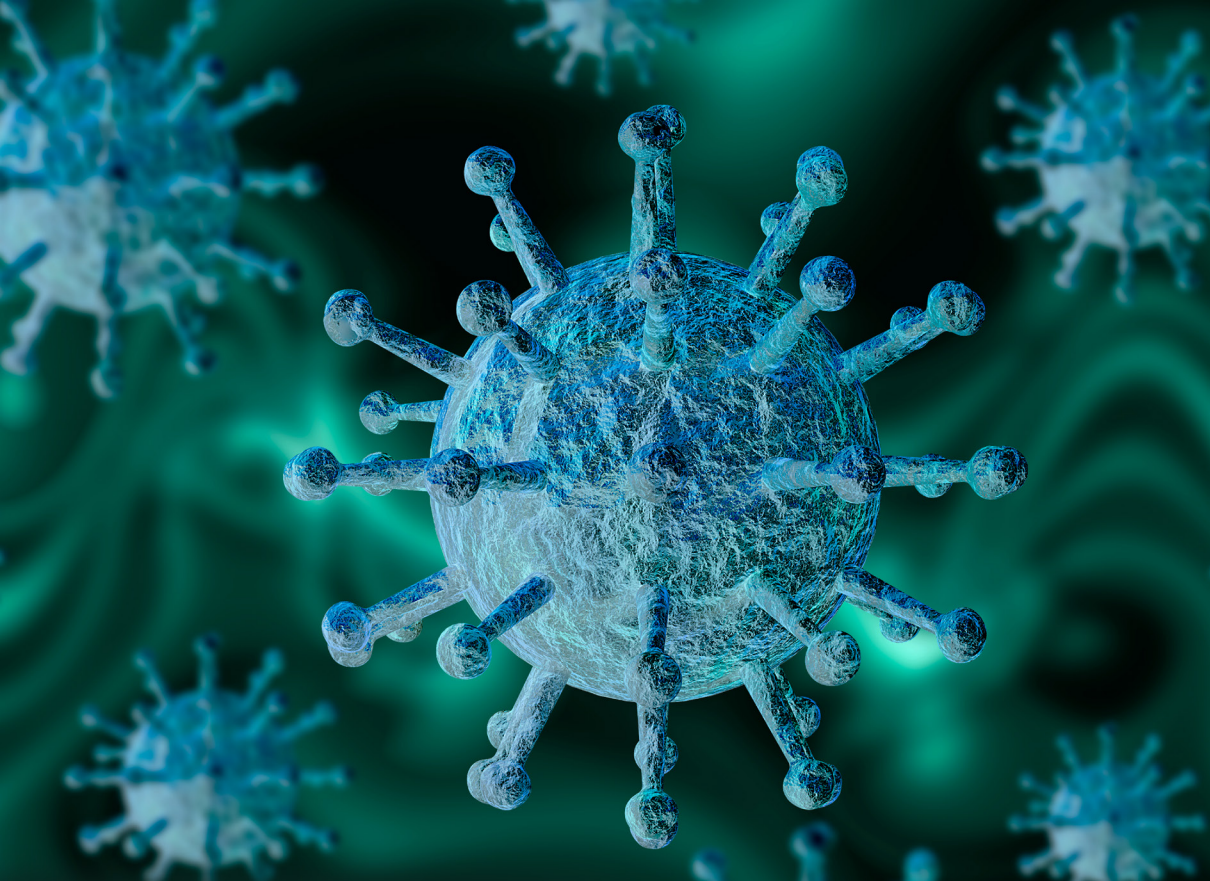

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