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Review

Antimicrobial potential of chitosan

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Chitosan is a biopolymer that has been used in the production of biomaterials and is the focus of numerous studies due to its antimicrobial activity. Chitosan has high value-added applications in medicine, agricultural and cosmetics. Its broad spectrum of action, strong bactericidal/bacteriostatic activity and low cytotoxicity are some of the many advantages of chitosan as an antimicrobial agent. This paper consists of a review of the previous studies of the antimicrobial properties of chitosan and its derivatives. Articles were searched in the databases "Pubmed", "Scopus" and "Web of Knowledge", and excluded those which used the combination of chitosan with some drug or active herbal principle, without restriction of year of publication. This research was carried out to investigate the impact factor of each journal and to develop a timeline to demonstrate the expansion of research in the area. Of the total of 52 articles found, 78.85% (41) dealt with the antibacterial activity of chitosan and 30.77% (16) with antifungal activity. Of the 41 articles that dealt with bacteria, 39.02% (16) evaluated the activity of chitosan against *Staphylococcus aureus* and 43.89% (18) against *Escherichia coli*. The antifungal activity was assessed in 37.5% (6) of strains of *Candida albicans*. It was observed that there has been an increasing use of chitosan as an antimicrobial agent in recent years, possibly related to its greater use in the production of polymer microparticles and microspheres in various areas. The average impact factor was 2.62 (± 1.10), by JCR, which indicates the rigorous selection of the articles used in this review and the greater credibility of this study. Although there are a growing number of studies on the antimicrobial properties of chitosan, further studies are needed to maximize its use. It is important to encourage research to evaluate the antimicrobial effect of chitosan on other clinical pathogens resistant to antimicrobial activity.

Key words: Chitosan, anti-bacterial agents, antifungal agents.

INTRODUCTION

The production of chitosan-based biomaterials has been the focus of numerous studies, since this biopolymer and its derivatives have proved to be promising agents in the treatment and prevention of various diseases because

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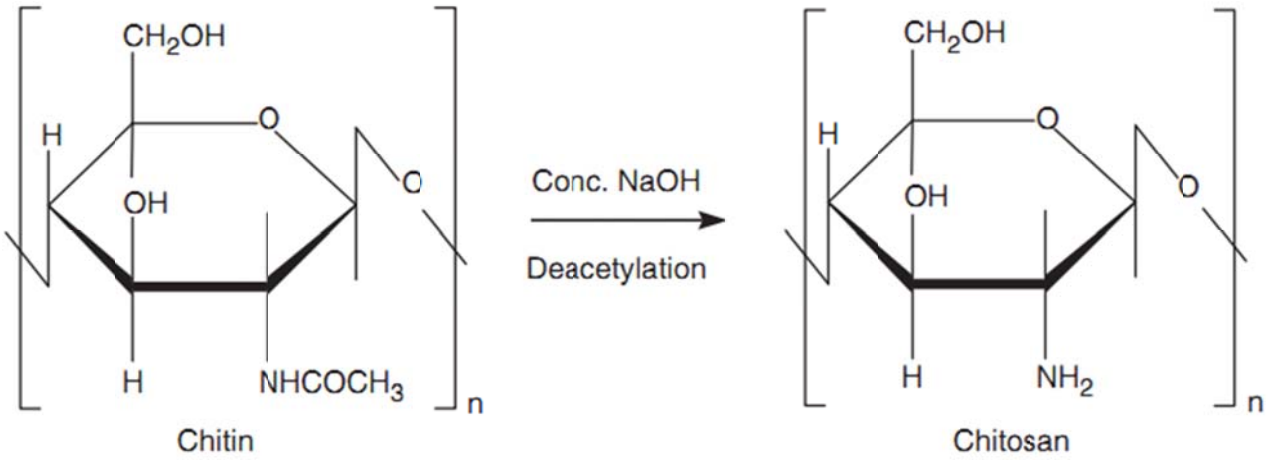

Figure 1. Chemical structure of chitin and chitosan after deacetylation. Adapted from Dutta et al. (2012).

they have numerous cellular actions (Kulikov and Shumkova, 2014); Aam et al., 2010). The term "chitosan" refers to the group of natural polycationic polysaccharides with high molecular weight and different viscosities, pKa and degrees of acetylation (Costa et al., 2014; Tsai et al., 2011; Raafat et al., 2008). It is produced by the deacetylation of chitin found in the exoskeleton of arthropods, crustacean shell, insects, algae and fungi (Tayel et al., 2010; Van der Mei et al., 2007; Prado et al., 2004). During its production, the degree of deacetylation should be monitored because this influences the antimicrobial polymer (Tayel et al., 2010). Figure 1 shows the chemical structure of chitin and chitosan.

Since 2013, the chitosan extracted from shrimp has been considered "generally recognized as safe" (GRAS) for use in food by the US Food and Drug Administration (Jeon et al., 2013). For Tayel et al. (2014) and Kumaresapillai et al. (2011), the chitosan produced and extracted from fungi is important for its low environmental impact, the possibility of control in certain conditions and large-scale production.

Chitosan has several characteristics that allow its application in widely different fields of science, these are: easy formation of gels, filmogenic capacity and good mechanical properties due to its physico-chemical characteristics (Kulikov and Shumkova, 2014), as well as being biodegradable, low allergenic biocompatibility potential and mucoadhesive properties, which are favorable in some of its applications (Patel et al., 2005). Due to its positive charge from the deacetylation, chitosan has important physiological and biological characteristics for the food, cosmetic, biomedical, pharmaceutical and agricultural industries (Badawy and Rabea, 2013; Tayel et al., 2010). It is used in the food industry to maintain the quality of many fruits, considering that in freshly harvested products there are the possibilities of increased contamination (Valle et al., 2012; Campaniello et al., 2008). It is used in the pharmaceutical industry to encapsulate compounds and to target drugs for resistant microorganisms to antibiotics, among other uses (Jeon et al., 2013).

The advantages of the use of chitosan as an antimicrobial agent are numerous and can highlight the broad spectrum of action with its strong bactericidal/ bacteriostatic activity and low cytotoxicity. However, despite having several advantages over chemical disinfectants, literature is occasionally contradictory regarding its antimicrobial activity (Costa et al., 2014; Dutta et al., 2012; Tayel et al., 2010). Therefore, this review aimed to bring together recent studies on the antimicrobial activity of chitosan and its derivatives showing possible causal factors in the different results.

ANTIMICROBIAL ACTIVITY OF CHITOSAN

The use of chitosan as an antimicrobial agent has increased in recent years (Tables 1 and 2). This increase may be related to the increase in studies on the production of polymer microparticles, and microspheres in various fields.

Antibacterial activity

Besides antibacterial activity, Ji et al. (2009a) realized chitosan stimulated proliferation of human periodontal ligament cells. There are still reports that chitosan influences the activation of the complement system, acts as an immune-potentializer to nonspecific activation of immune cells, accelerates the production of biological mediators and the infiltration of inflammatory cells (macrophages and polymorphonuclear) and has an effect on fibroblasts (Moon et al., 2007; Ueno et al., 2001). These findings are important to show that chitosan could be used in the treatment of periodontal diseases (Ji et al., 2009b), since it accelerates the inflammatory response

Table 1. Antibacterial activity of chitosan on specific bacteria, for study and source of chitosan.

Reference	Bacteria	Origin of chitosan
Badawy et al. (2014)	*Agrobacterium tumefaciens* *Erwinia carotovora*	Company (Sigma-Aldrich)
Costa et al. (2014)	*Enterococci* *Streptococci*	Company (Sigma-Aldrich)
Inta et al. (2014)	*Bacillus subtilis* *Escherichia coli* *Staphylococcus aureus*	Company (Seafresh Chitosan Lab Co.)
Jeon et al. (2013)	*Escherichia coli* *Klebsiella pneumoniae* *Salmonella enterica* *Streptococcus uberis* *Vibrio cholerae*	Company (Sigma-Aldrich)
Kulikov and Shumkova (2014)	*Staphylococcus aureus* *Staphylococcus epidermidis*	Crustaceans Animal (Crab)
Sahariah et al. (2014)	*Escherichia coli* *Staphylococcus aureus*	
Tayel et al. (2014)	Coliformes *Enterobacteriaceae* *Escherichia coli* *Staphylococcus aureus*	Fungal (*Aspergillus brasiliensis)*
Yang et al. (2014)	*Acidovorax avenae*	Crustaceans Animal (Caranguejo)
Younes et al. (2014)	*Escherichia coli* *Pseudomonas aeruginosa* *Klebsiella pneumoniae* *Salmonella typhi*	Company (Aber Technologies France)
Geng et al. (2012)	*Bacillus subtilis* *Salmonella cholerae suis*	Crustaceans Animal (Shrimp)
Li et al. (2013)	*Xanthomonas oryzae pv.oryzae* *Xanthomonas oryzae pv.oryzicola*	Crustaceans Animal (Crab)
Mansilla et al. (2013)	*Pseudomonas syringae*	Crustaceans Animal (Shrimp)
Chen and Chung (2012)	*Lactobacillus brevis* *Streptococus mutans*	Crustaceans Animal (Shrimp)
Giner et al. (2012)	*Bacillus cereus* *Enterobacter aerogenes* *Escherichia coli* *Salmonella entérica* *Staphylococcus aureus*	Crustaceans Animal (Shrimp)
Huang et al. (2012)	*Escherichia coli* *Staphylococcus aureus*	Crustaceans Animal (Shrimp)
Jiang et al. (2012)	*Enterococcus faecalis* *Escherichia coli* *Listeria monocytogenes* *Salmonella* spp. *Staphylococcus aureus* *Vibrio* spp	Company (Keumho Chemical)
Li et al. (2012)	*Acidovorax citrulli*	
Logesh et al. (2012)	*Escherichia coli* *Vibrio cholerae* *Salmonella typhi* *Staphylococcus aureus*	Fungal (*Roccella montagnei lichen)*

Table 1. Contd.

Reference	*Bacteria*	Origin of chitosan
Simunek et al. (2012)	*Bacteroides thetaiotaomicron* *Clostridium beijerinckii* *Clostridium paraputrificum* *Faecalibacterium prausnitzii* *Roseburia intestinalis*	Company (Medicol Co.)
Chung et al. (2011)	*Escherichia coli* *Salmonella typhimurium* *Shigella dysenteriae* *Staphylococcus aureus* *Listeria monocytogenes* *Bacillus cereus*	Crustaceans Animal (Shrimp)
Kumaresapillai et al. (2011)	*Escherichia coli* *Proteus vulgaris* *Pseudomonas aeruginosa* *Salmonella paratyphi-A* *Salmonella typhi*	Fungal (*Aspergillus niger*)
Lou et al. (2011)	*Burkholderia. seminalis*	Crustaceans Animal (Caranguejo)
Mellegard et al. (2011a)	*Bacillus cereus*	Company (Novamatrix)
Mellegard et al. (2011b)	*Bacillus cereus* *Escherichia coli*	Crustaceans Animal (Shrimp)
Tsai et al. (2011)	*Acinetobacter baumannii* MRSA *Pseudomonas aeruginosa* *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus pyogenes*	Company (Shin Era Technology)
Tayel et al. (2010)	*Bacillus subtilis* *Escherichia coli* *Pseudomonas aeruginosa* *Pseudomonas fluorescence* *Salmonella typhimurium* *Sarcinia lutea* *Serratia marcescens* *Staphylococcus aureus*	Fungal (*Mucor rouxii*)
Ballal et al. (2009)	*Enterococcus faecalis*	Company (Sigma-Aldrich)
Fernandes et al. (2009)	*Bacillus cereus*	Company (Sigma-Aldrich)
Ji et al. (2009a)	*Aggregatibacter actinomycetemcomitans* *Porphyromonas gingivalis* *Prevotella intermedia* *Streptococcus mutans*	Company (Putian Zhongsheng Weiye Co.)
Ji et al. (2009b)	*Porphyromonas gingivalis* *Prevotella intermedia*	Company (Dongying Guofeng Fine Chemical Co)
Lee et al. (2009)	*Vibrio vulnificus*	
Fernandes et al. (2008)	*Escherichia coli* *Staphylococcus aureus*	Company (Sigma-Aldrich)
Raafat et al. (2008)	*Staphylococcus aureus* *Staphylococcus simulans*	Company (Sigma-Aldrich)
Sarasam et al. (2008)	*Actinobacillus actinomycetemcomitans* *Streptococcus mutans*	Company (Sigma-Aldrich)

Table 1. Contd.

Reference	*Bacteria*	Origin of chitosan
Chung and Chen (2007)	*Escherichia coli* *Staphylococcus aureus*	Crustaceans Animal (Shrimp)
Kim et al. (2007)	*Escherichia coli* *Salmonella typhi*	Company (Iljin Pharmaceuticals)
Moon et al. (2007)	*Staphylococcus aureus*	Crustaceans Animal (Crab)
Anas et al. (2005)	*Vibrio cholerae* *Vibrio parahaemolyticus* *Vibrio mediterranei* *Vibrio nereis* *Vibrio proteolyticus* *Vibrio splendidus* *Vibrio vulnificus* *V*ibrio *alginolyticus*	Company (M/s South India Sea Foods)
Fujimoto et al. (2005)	*Escherichia coli* *Legionella pneumophila* *Staphylococcus aureus*	Company (Wako Pure Chemicals Co.)
Chung et al. (2004)	*Escherichia coli* *Pseudomonas aeruginosa* *Salmonella typhimurium* *Staphylococcus aureus* *Streptococcus faecalis*	Crustaceans Animal (Shrimp)
Rhoades and Roller (2000)	*Bacillus spp.* *Cryptococcus albidus* *Pseudomonas fragi*	Company (Pronova Biopolymer)

and the repair process, enabling the reestablishment of normal microbiota and repairing the periodontal ligament.

Bacteremia caused by *Staphylococcus aureus* is the most common cause of bloodstream infections associated with medical treatment (Hii et al., 2013) and *Escherichia coli* bacteremia is the third (Hii et al., 2013). Chung and Chen (2007) reported that chitosan can destroy the cellular structure of *E. coli* and *S. aureus*, leading to leakage of lysosomal enzymes and nucleotides in the cell.

However, other studies were inconclusive regarding the antimicrobial effect of chitosan. Although they observed antibacterial activity of chitosan in *E. coli* and *Legionella pneumophila*, Fujimoto et al. (2005) believed that, it is due to the decrease of the pH value of derivatives of the organic acids and not of the polymer.

The antibacterial activity of chitosan may be associated with a mechanism for sequential separation between the cell wall and cell membrane, followed by their destruction beyond the cell lysis and change in the osmotic pressure (Kulikov et al., 2014; Geng et al., 2012; Lou et al., 2011; Chen and Chung, 2012). For Tayel et al. (2010), chitosan tends to act in the peptidoglycan of cell wall.

Recently, Jeon et al. (2013) suggested that the antimicrobial activity of chitosan is partially mediated by the OmpA, an outer membrane protein of bacteria incorporated into a β-barrel structure, responsible for cell surface integrity. This contrasts with what has been proposed by Raafat et al. (2008), who proposed that there is no evidence that the antimicrobial activity of chitosan is mediated by direct action on the cell membrane.

There were also suggestions that the antibacterial effect of chitosan is related to its molecular weight, since the higher the molecular weight of the polymer, the greater the antimicrobial activity against Gram-positive bacteria. Whereas, for Gram-negative bacteria, the lower mass molecular chitosan implies greater antimicrobial activity (Lee et al., 2009).

Thus, the polymer has a greater effect on Gram-positive bacteria than on Gram-negative bacteria and some authors suggested that the positive charge present in the amino group of chitosan may interact with sites on the cell surface of the bacteria causing disturbances in cellular permeability (Younes et al., 2014; Chen and Chung, 2012; Chung et al., 2004; Helander et al., 2001), and changes in DNA and RNA (Tayel et al., 2010). It is also important to emphasize that chitosan may alter the presence of metal within the cell, inhibiting microbial growth and activating the host's immune response, which acts on the inhibition of various enzymes (Tayel et al.,

Table 2. Antifungal activity of chitosan on specific fungi for study and source of chitosan.

Reference	Fungus	Origin of chitosan
Badawy et al. (2014)	*Botryodiplodia theobromae* *Fusarium oxysporum* *Phytophthora infestans*	Company (Sigma-Aldrich)
Chaterjee et al. (2014)	*Macrophomina phaseolina*	Company (Sigma-Aldrich)
Elkholy et al. (2014)	*Rhizoctonia solani* *Sclerotium rolfsii*	Crustaceans Animal (Shrimp)
Kulikov et al. (2014)	*Candida albicans*	
Younes et al. (2014)	*Alternaria solani* *Aspergillus niger* *Fusarium oxysporum*	Company (Aber Technologies France)
Chien et al. (2013)	*Candida albicans*	Company (Shin Era Technology)
Pedro et al. (2013)	*Aspergillus flavus*	Company (Sigma Aldrich)
Giner et al. (2012)	*Aspergillus niger* *Candida albicans* *Penicillium digitatum* *Pichia anomala* *Pichia membranaefaciens* *Saccharomyces cerevisiae*	Crustaceans Animal (Shrimp)
Ing et al. (2012)	*Aspergillus niger* *Candida albicans* *Fusarium solani*	Company (Sigma-Aldrich)
Liu et al. (2012)	*Rhizoctonia solani*	Company (Sigma-Aldrich)
Sajomsang et al. (2011)	*Microsporum gypseum.* *Trichophyton mentagrophyte* *Trichophyton rubrum,*	Company (Seafresh Chitosan)
Li et al. (2010)	*Cladosporium cucumerinum* *Colletotrichum lagenarium* *Fusarium oxysporum* *Monilinia fructicola,*	Company (Qingdao Baicheng Biochemical Corp)
Ballal et al. (2009)	*Candida albicans*	Company (Sigma-Aldrich)
Guo et al. (2007)	*Botrytis cinerea* *Colletotrichum lagenarium*	Company (Qingdao Baicheng Biochemical Corp)
Peña et al. (2004)	*Candida albicans*	
Rhoades and Roller (2000)	*Candida* spp. *Saccharomyces cerevisiae* *Saccharomycodes ludwigii Rhodotorula* spp. *Zygosaccharomyces bailii*	Company (Pronova Biopolymer)

2010). Studies on the antimicrobial activity of pharmaceutical formulations containing chitosan were also carried out. The effectiveness of a chitosan-based mouthwash, used to combat oral bacteria, has been found to be similar to marketed mouthwashes, being a viable alternative for the industry and for the population (Chen and Chung, 2012), since the chitosan acts as a bactericidal agent and bacteriostatic (Fernandes et al., 2008).

Antifungal activity

Factors such as the pH of the solution used for diluting the chitosan, presence of bromine, chlorine and thiourea, the chemical structure, the degree of quaternization and the hydrophobic/hydrophilic balance influence the antifungal activity of the polymer (Elkholy et al., 2014; Sajomsang et al., 2011; Guo et al., 2007; Roller and Covill, 1999). Furthermore, the molecular weight was

directly proportional to the inhibition of fungal growth (Younes et al., 2014). Therefore, chitosan has the potential to be used as preservation medium for low pH foods which suffer from fungal infection (Rhoades and Roller, 2000).

The candidemia is a major concern for hospitalized patients because *Candida* species are the most important nosocomial pathogens, which can generate prolonged periods of hospitalization or even cause death. The prevalence of mortality from candidemia can reach up to 61% (Kreusch and Karstaedt, 2013). Although the *Candida albicans* can still mostly be responsible for these diseases, other species are increasing their role in candidemia (Wu et al., 2014; Karstaedt, 2013). *Candida tropicalis, Candida parapsilosis, Candida glabrata* and *Candida guilliermondii* are the most prevalent, just after *C. albicans* (Wu et al., 2014; Karstaedt, 2013). *C. albicans* is an opportunistic pathogen and the infections from this fungus represent a serious problem, especially for immunocompromised patients (Peña et al., 2004).

Studies on the action mechanism of chitosan on *C. albicans* have identified the severe alterations of the cell wall and the internal structure of the fungus, as well as the increase in the flow of potassium and calcium required for inhibition of respiration, fermentation and cell viability, which is responsible for the antifungal effect of the polymer (Kulikov et al., 2014; Peña et al., 2004). The increased cytoplasmic calcium flux causes changes in the cytoplasmic wall, leading to loss of cellular permeability and consequently cell death.

CONCLUSION

Although there are a growing number of studies on the antimicrobial properties of chitosan, more studies are needed to maximize the use of this polymer. It is important to encourage research that will evaluate the antimicrobial effect of chitosan on other pathogens of clinical interest, which have antimicrobial resistance.

Conflict of interests

The authors did not declare any conflict of interest.

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