ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 08, Issue, 02, pp.18740-18743, February, 2018

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

ANTIMICROBIAL PROPERTIES OF CHITOSAN- A REVIEW

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ARTICLE INFO

Article History:
Received 15th November, 2017
Received in revised form
22nd December, 2017
Accepted 19th January, 2018
Published online 28th February, 2018

Key Words:
Chitosan,
Antimicrobial activity,
Food preservatives.

ABSTRACT

Chitosan is a versatile hydrophic polysaccharide derived from chitin, has a broad antimicrobial spectrum to which gram negative, Gram positive bacteria and fungi are highly susceptible. The applicability of the chitosan is directly with its physicochemical features. Without affect its chemical properties different process of extraction and purification can be caused changes in deactylation degree, molecular weight, thermal stability and crystalline level in the chitosan molecule. Regarding its antimicrobial activity, chitosan has low toxicity and the development of resistance has being reported. This review aimed to create an awareness among the public for the utilization of chitosan and it's application values for future.

Citation: Reena, T., Ajusha Suresh and Saraniya, S.S. 2018. "Antimicrobial properties of Chitosan- A Review", *International Journal of Development Research*, 8, (02), 18740-18743.

INTRODUCTION

Chitin and chitosan are natural polymer composed by units of 2-amino-2 deeoxy-D-glycopyranase and of 2-acetamidc 2-dcoxy- D-glycopyranosc interconnected by glycosidic bonds p- 1-4 chitosan is found in the cell wall of fungi, mainly of the order Mucorales (Assis *et al*., 2003; Assis and Silva, 2003). Chitosan is a polysaccharide largely distributed in nature and is mainly found in the exoskeleton of crustaceans, insects, nematodes and in cell wall of yeast and fungi (Canella and Carcia, 2001; Signini, 2002). It is characterized by a fibrous structure. Crustaceans like shrimp, crabs and loster arc abundantly padded with chitosan. The chemical structure of chitin is similar to cellulose. It has one hydroxyl group on each monomer substituted with an acetylamine group. Extraction of chitin involves demoralization (removal of calcium carbonate), it is generally done by hot reaction with HCL, HNO3 etc. followed by an deproteinzation. Chitosan is insoluble in water, but soluble in dilute aqueous acidic solutions below its PKa (-6.3). Hydrogen bonds links the chains of those polymers in there different automorphic structures, this showing polymorpohism and configuration. They mainly shows conformations such as α, β and γ (Winterowd *et al*., 2014; Pillai *et al*., 2009). Of these α form is most common found in the cell wall of fungi, carapace of crustaceans and in the cuticle of some insects. α confirmation shows anti parallel arrangement in which chains are arranged in opposite direction. β configuration shows parallel forms and the third form γ is characterized by three units of chains. Antimicrobial activity of chitosan has been demonstrated against many bacteria, filamentous fungi and yeasts, chitosan exhibits wide spectrum activity and shows high microbicidal activity against Gram positive and Gram negative bacteria and shows lower toxicity towards mammalian cells Antimicrobial activity of chitosan relies on several intrinsic and cxtrinisic factors such as pH, microorganism species, molecular weight, PKa, degree of dcacetylation (DO) of chitosan etc.. Many approaches have been used for studying the antimicrobial activities of chitosan. Chitosan has tremendous advancements in molecular biological, nanotechnology, pharmaceutical, agricultural level etc (Zivanovic *et al*., 2004).

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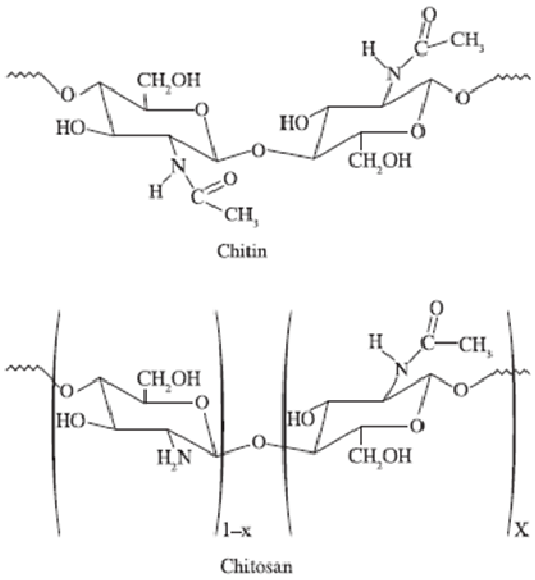

Figure 1. Schematic representation of the chemical structures of the chitin and chitosan

Antimicrobial models of chitosan

The interaction of these microorganisms with chitosan occurs in its different forms such as solutions, tablets, micronano particles, granules, film, gels, hydrogels etc., Chitosan is considered to be a bactericidal (Kill the live bacteria) or bacteriostatic (hinder the growth of bacteria). Several factors contribute the antibacterial action (Kumar *et al*., 2005). Three models have been proposed and among these the most acceptable one being the interaction between positively charged chitin or chitosin molecules and negatively charged microbial cell membranes.

The interaction is mainly mediated by the electrostatic force between the protonated NH3+ group and the negative residues (Raafat and Bargen, 2008) on the membrane surface (Tsai and Su, 1999). It was possible to observe and identify chitosan molecules attached on bacterial cell surfaces. Chitosan molecules are found attached on bacterial cell surfaces. In the interacting sites it was found that the cell membrane became locally detached from the cell wall, which give rise to vacuole-like structure underneath the cell wall.

This attachment generates ions and water efflux and thus decreasing the internal bacterial pressure (Kumar *et al*., 2005) and results in the conformational change on the cell membrane which finally results in the lysis of the Gram positive and Gram negative bacteria (Young and Kauss, 1983; Chug and Chen, 2008; Eaton *et al*.,). Since this mechanism is based on electrostatic interaction, the greater the number of cationized amines the higher will be the antimicrobial activity (Helender *et al*., 2004; Masson *et al*., 2008). The bacterial effectiveness on Gram positive and Gram negativce is however controversial Some studies shows that the chitosan generally shows stronger effects for Gram positive bacteria (Eg. *Bacillus ceres, Etegillus megaterium,. Staphlococcus aureus. Lactobacillus planetarium, Lactobacillus brevis, Lactobacillus bulgaris) than for Gram negative bacteria(Escherichia coli, Salmonella typhimuriurn,. Pseudomonas fluorescence)*. Gram positive organism shows more sensitive to chitosan due to its hydrophilicity than Gram negative bacteria. Another mechanism shows that the binding of chitosan with microbial DNA. This leads to the inhibition of the mRNA and protein synthesis through the penetration of chirosan into the nuclei of the microorganisms (Yalpani *et al*., 2009; Haduriger *et al*., 1981; Sudarshan *et al*., 1992). The thirdmechanism deals with the chelation of metals and it is well known chitosan has excellent metal binding capacities.

Table 1. Application of antimicrobial property of chitosan

Support (Preparation method)	Application	Tested microorganisms
Chitosan acetate	Food preservative	*Escherichia coli Staphylococcus aureus*
Chitosan and its maillard reaction products	Food preservative	*Bacillus subtilis*
Chitosan-hydroxy propyl methyl cellulose film	Packaging materials	*Listeria monocytogenes*
Chitosan/polyethylene oxide film	Packaging materials	*Escherichia coli*
Chitosan-nylon-6/Ag blended membranes	Packaging materials	*Escherichia coli Staphylococcus aureus*
Polyproylcne/Chitosan/ Pectin films	Packaging materials	*Clavibactcr michiganensis Pseudomonas solanacearum Fusarium oxysporum Verticillium alboatrum*
Chitosan hydroxyl propyl methyl cellulose film	Edible films and coating	*Aspergillus niger*
Chitosan	Food additive	*Staphlococcus*
Alginate / chitosan fibres	Wound drowing materials	*Staphylococcus aureus*
Quaternised chitosan nanofibres	Wound healing application	*Escherichia coli Staphylococcus aureus*
Polyacrylonitrile/Chitosa/heparin	Haemodialysis	*Aurococcus*
Carboxymethyl lechitosan N-O So_4	Drugs for AIDS	HIV I
Water soluble carboxyl methyl Chitosan	Cotton fabric	*Esc Staphylococcus aureus herichia coli*
Poly (n-butyl acrylate) cores and chitosan shells core-shell particles	Cotton fabric	*Staphylococcus aureus*

Chitosan						
					Antimicrobial	
Origin	Form	DA (%)	MW	solubility	C1M	CBM
Crustanceans	GEL	65	$324X10^5$	Acetic acid 1%	1.25	2.50
Crustanceans	GEL	90	$324X10^5$	Acetic acid 1%	1.25	2 50
Crustanceans	Solution	85	$324X10^5$	Water-chilosan Hydrochloride	0.06	0.03
Fungi	GEL	85	2 7 2 X 1 0	Acetic acid 1%	1.25	2.00
Fungi	GEL	85	$314XJ0^4$	Acetic acid 1%	0.06	1.25
Fungi	Solution	85	$314X10^4$	Water chitosan Hydrochloride	0.03	0.01
Fungi	Solution	85	$236X10^5$	Water chitosan Hydrochloride	0.06	0.03
Fungi	Solution	85	$272X10^5$	Water chitosan Hydrochloride	0.06	0.03

The amine groups in the chitosan molecules are responsible for the uptake of metal cations by chelation. It is unquestionable into the cell and contributing to cell death (Kumar *et al*., 2005).

Mechanism of antimicrobial activity of chitosan against bacteria

It is assumed that chitosan acts in the cell wall of the microorganism there by modifying the electric potential of the cellular membrane (Stamford *et al*., 2011). According to Kong *et al*., regarding the antimicrobial activity, chitosan show low toxicity and the resistance development has not occurred. Variations in chitosan's bactericidal efficacy arise from various factors such as Microbial factors (microbial species, age of the cell) *Intrinisic* factor of the chitosan (positive change density, molecular weight, hydrophilic abd hydrophobic characteristics, chelating capacity) physical state factors (soluble and soild state) and Environmental factors (pll, ionic forces, temperature, time). The antimicrobial action mechanism of the chitosan is not fully elucidated. Some studies evidenced that the amino groups of the chitosan when it contact with the physiological fluids are promoted and when bind to anionic groups of the microorganism, resulting in the agglutination (Senel *et al*., 2000; Avadi *et al*., 2004) of the microbial cells and inhibition of the microbial growth. Yadav, Bhisc (2004) reported that when chitosan interact with the bacterial cell the chitosan promotes the displacement of Ca++ of the anionic sites of the cell surface resulting in cell damage. Other studies shows that the positive charge of the chitosan and the negative charge of the microbial cell wall leads to the cell rupture and loss of important constituent of the microbial life.

Chitosan with low molecular weight penetrates in the cell and it links with the microbial DNA thereby inhibiting the transcription and consequently the translation. Whereas, chitosan with high molecular weight act as a chelant agent, binding to the cell membrane (Pedro *et al*., 1994). The Gram negative bacteria posses the external membrane the cell wail composed of lipopolysacharides that provide a hydrophilic surface to the bacterium. The polysaccharides have anionic group (phosphate, carboxyl), which contributes stability of lipopolysacharides through the divalent electrostatic interaction with cations. The removal of those cations results in the release of the lipopolysacharides. On contrast, the Gram positive bacterial cell wall is composed of pcptidoglycon and teichoic acid These components covalently links to the acid N-acetylmuramic of the peptidoglycon through glycolipid, which provides binding site for chitosan, causing functional riots in the membrane (Yadav and Bhinse, 2004; Senel *et al*., 2000; Avadi *et al*., 2004; Pedro 1994; Shibasaki *et al*., 1994).

Antifungal activity

Similar top bacteria, chitosan shows antifungal property. Chitosan acts as fungistatic rather than fungicidal. Chitosan shows high inhibitory effect against spore germination, radial growth, germ tube elongation (Ghaowth *et al*., Sashai and Manocha, 1993) etc.. Many studies have conducted on fungi associated with the plant and food spoilers. The antifungal mechanism is carried out by cell wall morphogenesis with chitosan, thereby interfering the fungal growth as in bacteria. The antifungal activity of chitosan depends upon the concentration of DA and local pH.

Chitosan

The ideal antimicrobial polymer should posses the following charactcrstics

- Easily and inexpensively synthesized.
- Biocide to broad spectrum of pathogenic microorganism in brief time of contact.
- Donot decompose or emit toxic products.
- Stable in long-term usage and storage at the temperature of its indendent application.
- Shouldnot be toxic or irritating to those who are handling it.
- Can be regenerated upon loss of activity.
- Not soluble in water for a water-disinfectant application (Kcnawy ct al, 2007).

As a natural polysaccharide chitosan possess many of these attributes. From the biological stand point, chitosan and its derivatcs are very attractive for medical, food and textile industries. Chen et .al; 2005, a, b. 2006; Deng et .al; 2007; Fan *et al*; 2006; Fuetal, 2005; Fujita et .al; 2004; Gama Sosa *et al*., 1991; Gupta *et al*., 2007; Hayashi, *et al*., 2004; Li, *et al*., 2007; Lin *et al*., 2004; Ma *et al*., 2008; Maher *et al*., 2008; Moiier *et al*., 2004; Sadeghi *et al*., 2008; Sebti *et al*., 2007; Wu *et al*., 2004; Yang and Lin, 2004; Yang *et al*., 2004, 2008; Ye *et al*., 2005; Yu *et al*., 2006; Zivanovie *et al*., 2007. Table:2 Studies in vitro performed by the Karyology research group Microbiology laboratory form UFPE, demonstrating the minimum inhibitory concentration (MIM) and bacterial concentration (CBM) of the chitosan and its derivativs from different origins, varying the parameters deacetylation level molecular weight and solubility to *Staphylococcus mutans.*

Conclusion

Chitosan is a versatile material with proved antimicrobial activity. Three antimicrobial mechanism have been proposed.

a) The ionic surface interaction resulting in cell wall leakage.
b) The inhibition of the mRNA and protein synthesis.
c) Formation of an internal barrier provoking the suppression of essential nutrients to microbial growth.

Through this review, we would like to conclude that chitosan which is found mainly in the exoskeleton of crustaceans, insects, nemetodes and in all cell wall of yeast and fungi are widely used in many applications such as industrial, agricultural, food industries etc., the antimicrobial activity of chitosan is a considerable interest and attention over the last decads.

Acknowledgement

Here the authors express their sincere gratitude to the Management and the Department for their support and engaragement.

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