International Journal of Food Microbiology 144 (2010) 51–63

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Review

Antimicrobial properties of chitosan and mode of action: A state of the art review

Ming Kong [a,b,1], Xi Guang Chen [a,1], Ke Xing [c], Hyun Jin Park [b,*]

[a] College of Marine Life Science, Ocean University of China, Qingdao, 266003, Shandong, PR China
[b] School of Life Sciences and Biotechnology, Korea University, Seoul, 136-701, South Korea
[c] College of Life Science, Xuzhou Normal University, Xuzhou, 221116, Jiangsu, PR China

ARTICLE INFO

Article history:
Received 7 June 2010
Received in revised form 26 August 2010
Accepted 16 September 2010

Keywords:
Chitosan
Antimicrobial activity
Mechanism
Application

ABSTRACT

Owing to its high biodegradability, and nontoxicity and antimicrobial properties, chitosan is widely-used as an antimicrobial agent either alone or blended with other natural polymers. To broaden chitosan's antimicrobial applicability, comprehensive knowledge of its activity is necessary. The paper reviews the current trend of investigation on antimicrobial activities of chitosan and its mode of action. Chitosan-mediated inhibition is affected by several factors can be classified into four types as intrinsic, environmental, microorganism and physical state, according to their respective roles. In this review, different physical states are comparatively discussed. Mode of antimicrobial action is discussed in parts of the active compound (chitosan) and the target (microorganisms) collectively and independently in same complex. Finally, the general antimicrobial applications of chitosan and perspectives about future studies in this field are considered.

Contents

* Corresponding author.
E-mail address: hjpark@korea.ac.kr (H.J. Park).
[1] This author and first author are equal in contribution to this paper.

0168-1605/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2010.09.012

1. Introduction

Chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose] is a collective name for a group of partially and fully deacetylated chitin compounds (Tikhonov et al., 2006). Due to its unique biological characteristics, including biodegradability and nontoxicity, many applications have been found either alone or blended with other natural polymers (starch, gelatin, alginates) in the food, pharmaceutical, textile, agriculture, water treatment and cosmetics industries (Arvanitoyannis et al., 1998; Arvanitoyannis, 1999; Haque et al., 2005; Kim et al., 2005; Roberts, 1992; Yamada et al., 2005). Antimicrobial activity of chitosan has been demonstrated against many bacteria, filamentous fungi and yeasts (Hirano and Nagao 1989; Kendra and Hadwiser, 1984; Uchida et al., 1989; Ueno et al., 1997). Chitosan has wide spectrum of activity and high killing rate against Gram-positive and Gram-negative bacteria, but lower toxicity toward mammalian cells (Franklin and Snow, 1981; Takemono et al., 1989). Ever since the broad-spectrum antibacterial activity of chitosan was first proposed by Allen (Allan and Hardwiger, 1979), along with great commercial potential, the antimicrobial property of chitosan and its derivatives have been attracting great attention from researchers.

Investigation of the antimicrobial properties of chitosan has been a long journey of scientific exploration and technological development. The journey began two decades ago, with studies on the biological phenomena arising from foodborne and soilborne pathogenic fungi in the food and agriculture industries (Rabea et al., 2003). In light of their intimate relationship with human activities, bacteria rightly began to receive more attention in the search for efficacious antimicrobials. The studies at that time were typically carried out via chemical, biochemical, microbiological and medical assays of chitosan and its derivatives. In some cases, but rarely so, molecular and cell approaches were utilized. The outcomes obtained through this period suggested that antimicrobial activities of chitosan and its derivatives relied on numerous intrinsic and extrinsic factors, such as pH, microorganism species, presence or absence of metal cations, pKa, Molecular weight (Mw) and degree of deacetylation (DD) of chitosan, etc. Some basic hypotheses about underlying antimicrobial mechanisms were also proposed (Zivanovic et al., 2004). Based on the outcomes, various antimicrobial agents based on chitosan or its derivatives emerged. At the same time, since biocide resistant bacteria and fungi, growing public health awareness of pathogenic microorganism raised demands for safe and efficacious agents that were less prone to stimulating development of resistance. In addition to tremendous advancements in molecular biological, pharmaceutical, cell biological technologies and detecting methods, nanotechnology emerged and began playing an extraordinary role, carrying the potential to extend antimicrobial treatment to the atomic level.

The many approaches that have been used in studying antimicrobial activities of chitosan and its derivatives have given rise to various physical forms of chitosan in differing methods, from the original solution applied in agriculture, to film structure in food sector and to ubiquitous pharmaceutical nanostructure materials. Different physical states of chitosan, as a crucial factor influencing antimicrobial activity, are supposed to have strongly considered but always being underestimated. Chitosan's water-solubility casts important impact on its particular antimicrobial activities, and the relevant researches have accordingly attracted understandable attention in the water solution. In contrast, solid state research has been confining to the application of antimicrobial properties such as beads, films, fibers, and hydrogels, mostly aimed at biomedical applications (Kong et al., 2008a). Little attention has been paid to systemic investigations of the inhibitory effect in solid state, needless to its mode of action.

Based on the current situation of research and progress in corresponding areas, this review attempts to sum up the general developments in the study of antimicrobial properties of chitosan. Comparison of the antimicrobial activity between different physical states of chitosan is made, especially the solid form. Differences among influencing factors and corresponding modes of action are discussed in detail. Finally, present and potential future applications are discussed.

2. Investigation on antimicrobial activity of chitosan and its derivatives

Variations in chitosan's bactericidal efficacy arise from various factors. According to roles playing, these factors can be classified into four categories as follow: (1) microbial factors, related to microorganism species and cell age; (2) intrinsic factors of chitosan, including positive charge density, Mw, concentration, hydrophilic/hydrophobic characteristic and chelating capacity; (3) physical state, namely water-soluble and solid state of chitosan; (4) environmental factors, involving ionic strength in medium, pH, temperature and reactive time.

2.1. Microbial factors

2.1.1. Microbial species

Although owning a broad spectrum of antimicrobial activity, chitosan exhibits differing inhibitory efficiency against different fungi, Gram-positive and Gram-negative bacteria. Chitosan exerts an antifungi effect by suppressing sporulation and spore germination (Hernandez-Lauzardo et al., 2008). In contrast, the mode of antibacterial activity is a complicating process that differs between Gram-positive and Gram-negative bacteria due to different cell surface characteristics. In several studies, stronger antibacterial activity was apparent against Gram-negative bacteria than Gram-positive bacteria (Chung et al. 2004; No et al., 2002), while in another study Gram-positive bacteria were more susceptible, perhaps as a consequence of the Gram-negative outer membrane barrier (Zhong et al., 2008). Still many workers demonstrated there were no significant differences observed between the antibacterial activities against the bacterium (Wang et al., 2004). Various initial reaction materials and conditions contribute to the diverse consequences. Based on the available evidences, bacteria appear to be generally less sensitive to the antimicrobial action of chitosan than fungi. The antifungal activity of chitosan is greater at lower pH values (Roller and Covill, 1999).

2.1.2. Cell age

For a given microbial species, age of the cell can influence antimicrobial efficiency. For example, *S. aureus* CCRC 12657 in late-exponential phase are the most susceptible to lactose chitosan derivative with no viability evident after 10 h of incubation. Meanwhile, a relatively less population reduction in viable cells of 3.75 and 3.96 log cfu/mL, respectively, was observed with cells in the mid-exponential phase and late-stationary phase (Chen and Chou, 2005). It is suggested that the differences of cell surface electronic negativity vary with the phase of growth, which can lead to the differences in the susceptibility of cells towards chitosan (Tsai and Su,

1999). In contrast, *E. coli* O157:H7 in mid-exponential phase were the most susceptible, while stationary phase cells were the least susceptible to maltose chitosan derivative (Yang et al., 2007). The discrepancies were attributable to the different microorganisms examined, since the surface charge of microbial cells also varied with the microorganism (Bayer and Sloyer, 1990).

2.2. Intrinsic factors of chitosan

2.2.1. Positive charge density

Tremendous literatures support the essential importance of polycationic structure in antimicrobial activity. A higher positive charge density leads to strong electrostatic interaction. Therein, the positive charge is associated with DD or degree of substitution (DS) of chitosan or its derivatives, which affect positive charge density.

To some extent, chitosan microspheres with a high DD (97.5%) lead to higher positive charge density, which confers stronger antibacterial activity than moderate DD (83.7%) against *Staphylococcus aureus* at pH 5.5 (Kong et al., 2008b). One study reported that a higher DD with more positive charge was especially successful in inhibiting the growth of *S. aureus*, suggesting antibacterial activity of chitosan towards *S. aureus* enhanced with increasing DD (Takahashia et al., 2008).

Concerning chitosan derivatives, antimicrobial activity mostly depends on DS of the grafting groups. Investigation of the antibacterial activities of water-soluble N-alkylated disaccharide chitosan derivatives against *Escherichia coli* and *S. aureus* revealed that the antibacterial activity of chitosan derivatives is affected by the DS of disaccharides and the type of disaccharide present in the molecule (Yang et al., 2005). The same study suggested that, irrespective of the kind of disaccharide linked to the chitosan molecule, a DS of 30–40%, in general, produced the most pronounced antibacterial activity against *E. coli* and *S. aureus*, and that both microorganisms were most susceptible to cellobiose chitosan derivative DS 30–40% and maltose chitosan derivative DS 30–40%, respectively, among the various chitosan derivatives examined. The authors surmised that DS changed the pKa of the chitosan molecules, which made protonation of chitosan derivative molecules different from native chitosan under similar pH condition, favoring higher cation density.

The chitosan metal complex is another example regarding positive charge. A thiourea chitosan-silver ion (Ag^+) complex was prepared through the reaction of chitosan with ammonium thiocyanate in ethanol. The complex overcame the instability of Ag^+ and strengthened the antibacterial capacity by 20-folds compared with chitosan alone (Chen et al., 2005a,b). Similarly chitosan-zinc (Zn) complex showed a wide spectrum of effective antimicrobial activities (Wang et al., 2004), which were 2–8 times and 4–16-folds higher than those of chitosan and zinc sulfate, respectively, and improved with increasing content of zinc ions. The chitosan-Zn complexes exerted a more effective antibacterial activity equally against *E. coli* and *Corynebacterial* (minimal inhibitory concentration, MIC, of 0.000313%, Chitosan-Zn%, w/v) than its antifungal activity (Table 1) (Wang et al., 2004).

2.2.2. Mw

Numerous studies on bactericidal activity of chitosan have generated equivocal results concerning correlation between bactericidal activity and chitosan Mw. Some studies reported increasing chitosan Mw lead to decreasing chitosan activity against *E. coli*, while in other studies high Mw (HMw) chitosan displayed greater activity than low Mw (LMw) chitosan. In addition, activities still were found to be equal against *E. coli* and *Bacillus subtilis* regardless of Mw (Tikhonov et al., 2006).

Even though the limited available results on bactericidal activity of LMw chitosan were comparable depending on bacteria strains, conditions of biological testing and respective chitosan Mw, the results are not accordant with each other. For instance, 9.3 kDa chitosan inhibits growth of *E. coli* while 2.2 kDa chitosan promotes its growth (Tokura et al., 1997). As well, LMw chitosan (4.6 kDa) and its derivative showed better activity against bacteria, yeast and fungi (Tikhonov et al., 2006).

Table 1
The antibacterial activity of chitosan, $ZnSO_4$ and CS-Zn(c). Modified from Wang et al., 2004.

Microorganisms	Chitosan (CS%, w/v)	Chitosan-Zn complex (CS-Zn%, w/v)
Gram-negative bacteria		
Escherichia coli	0.025	0.00313
Pseudomonas aeruginosa	0.0125	0.00625
Proteus mirabilis	0.025	0.00625
Salmonella enteritidis	0.05	0.00625
Enterobacter aerogenes	0.05	0.00625
Gram-positive bacteria		
Staphylococcus aureus	0.05	0.0625
Corynebacterium	0.025	0.0313
Staphylococcus epidermidis	0.025	0.0125
Enterococcus faecalis	0.05	0.0125
Fungi		
Candida albicans	0.1	0.1
Candida parapsilosis	0.1	0.05

2.2.3. Hydrophilic/hydrophobic characteristic

Irrespective of their form or quantity, antimicrobial agents typically require water for activity. Totally dry samples are virtually incapable of releasing their energy stored in chemical bonds to initiate interaction. Hydrophilicity and hydrophobicity are conceptions also based upon water ambience, upon which the manner of antimicrobial interaction of chitosan is determined.

The hydrophilic characteristics of chitosan profoundly determine water solubility. The use of chitosan is limited by the compound's poor solubility in water (Dutta et al., 2004). Chemical modifications as an approach are efficient in improving the water solubility of chitosan and its derivatives, and widening their applications (Xie et al., 2007). The creation of water-soluble chitosan and its derivatives has been a central goal of investigations of antimicrobial activity, which have included saccharization, alkylation, acylation, quaternization and metalization. As one example, quaternary ammonium chitosan can be prepared by introducing quaternary ammonium group on dissociative hydroxyl group or amino group. In one study, quaternarized chitosan derivatives exhibited stronger antibacterial activity, broader spectrum and higher killing rate in comparison to chitosan (Ignatova, et al., 2006). This does not necessarily mean that quaternized chitosan is bound to exhibit a more pronounced inhibitory effect. One study reported that introductions of N, N-dimethylaminobenzyl and N-pyridylmethyl group into chitosan backbone did not confer antibacterial activity against *S. aureus* (Sajomsang et al., 2007). Although the total degree of quaternization was higher than that of N,N,N-trimethylammonium chitosan chloride, the antibacterial activity of the corresponding methylated chitosans against *S. aureus* failed to dramatically increase. This study suggested that a higher extent of N-substitution of N, N-dimethylaminobenzyl substituents significantly changed the hydrophilic/hydrophobic balance and reduced the potential of interaction between these derivatives and bacterial cell wall.

It is quite reasonable that the antimicrobial activity is dependent on the spacer length due to the change in both conformation and charge density of the polymer, which consequently affect the mode of interaction with the cytoplasmic membrane (Kenawy et al., 2007). For chitosan and its derivatives, the hydrophilic–lipophilic variation influences the antimicrobial properties. The hydrophobic characteristic of N-acylated chitosan can be favorable for the interaction of polymer molecule and bacterial cell, where the hydrophobicity of NHCS0.5 (N-hexanoyl chitosans, corresponding to a molar ratio of 0.5 compared with chitosan residue) is likely to be a contributing factor

for its enhanced inhibitory effect (Hu et al., 2007a,b). In another study, the presence of a long aliphatic chain facilitated the absorption and enhanced effect of a substituted LMW chitosan, N-/2(3)-(dodec-2-enyl)succinoyl/chitosans, onto cell walls via hydrophobic interaction with cell wall proteins (Tikhonov et al., 2006). In addition, the introduction of acyl groups improved the inhibition of *S. aureus* and the length of the acyl groups influenced the antibacterial activity (Huang et al., 2004). N-hexanoyl chitosan sulfates showed lower optical absorbance at 620 nm comparing with N-propanoyl chitosan sulfate, meaning the growth of *S. aureus* was more effectively inhibited.

Resembling that of *S. aureus*, in the same study, the inhibition of the growth of *E. coli* was also affected by the DS and the length of the acyl groups. The improvement of the antibacterial activities caused by acyl groups somehow might result from the hydrophobicity of the acyl sulfate. N-hexanoyl chitosan sulfate, which had a higher hydrophobicity, displayed greater inhibitory effect than N-propanoyl chitosan sulfate (Huang et al., 2004). Consistently, chitosan microspheres (CMs) grafted with oleoyl more potently inhibited the growth of *E. coli* than acetylated CMs (Kong et al., 2008a).

2.2.4. Chelating capacity

Chitosan possesses high chelating capacity for various metal ions (including Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Mg^{2+} and Cu^{2+}) in acid conditions, and it has been widely applied for the removal or recovery of metal ions in different industries (Kurita, 1998). Metal ions that combine with cell wall molecules of microorganism are crucial for stability of the cell wall. Chitosan-mediated chelation of such metal ions has often been implicated as a possible mode of antimicrobial action (Rabea et al., 2003). Not only does chelation play a part in acid condition, it is also able to combine divalent metal ions in neutral condition (Kong et al., 2008a). Additionally, via chelating capacity, chitosan metal complex is prepared and exerts strong antimicrobial activity as discussed above.

2.3. Physical state

Antimicrobial activity of chitosan is the result of series of reactions, rather than the cause of the reactions. The reactions take place between molecules of chitosan and cell wall, what is easy to interpret the morphology of molecules is responsible for the reactions efficiency. Equally, physical state of chitosan, upon which the existing morphology of molecules depends, acts a decisive role in its antimicrobial activity. Nonetheless, scant focus has been paid to the influence of different physical state.

2.3.1. Antimicrobial activity in soluble state

Soluble chitosan existing as a disassociating form in solution has an extending conformation, which enable reaction with the counterparts to a sufficient degree and bring the potential to full play (Phaechamud, 2008). This explains why soluble chitosan and its derivatives are more effective in inhibiting bacterial growth. According to the literatures (Chung et al., 2005; Xie et al., 2007), the minimal inhibitory concentration (MIC) of chitosan derivatives are significantly decreased against all tested bacteria than those of native chitosan. Meanwhile, owing to a sufficient touch with solution, soluble chitosan and its derivatives are readily affected by outer environmental factors as well as many intrinsic factors.

In one study, chitosan derivatives (chitosan and maltose, glucose, fructose, glucosamine) produced through the Maillard reaction enhanced the solubility of the native chitosan. Among them, chitosan-glucosamine derivative appeared to be more effective than other chitosan or chitosan derivatives as a natural bactericidal agent (Chung et al., 2005). Quaternary ammonium chitosan is another major example to improve solubility of chitosan by introducing hydrophilic groups into molecule. After quarternization, derivates exhibited better water solubility and stronger antibacterial activity as compared to chitosan (Xie et al., 2007). In addition, Mw and DD of chitosan may share the roles in terms of soluble chitosan. For instance, reducing the Mw of chitosan markedly heightens solubility. Alternation of Mw changes the content of N-acetylglucosamines units in chitosan, which will have intramolecular as well as intermolecular influence, resulting in different conformations of chitosan. However, improving solubility by controlling deacetylation comes at the cost of low yield (Kurita et al., 1991).

pH is also an important parameter. For soluble chitosan, pH is a crucial factor relating to solubility, and can further alter antimicrobial activity. Another pH effect is protonation of chitosan and its derivatives. Antimicrobial activity of chitosan and its derivatives is exhibited only when the pH is below the respective pKa, the value at which the soluble molecule could be disassociated as ions in solution. This mechanism is not restricted to soluble chitosan, but extends to solid chitosan.

2.3.2. Antimicrobial activity in solid state

Compared with soluble chitosan, rather than the extending conformation contact to solution, solid chitosan only get into touch with solution through surface, such as fibers, membrane, hydrogels, microspheres and nanopaticles. Hydrogels can be formed by covalently cross-linking chitosan with itself. Recently, many attempts have been made to create chitosan particulate systems that could form dispersion in solution with considerable reactive surface area. The shift of physical state is sure to bring variation of its antimicrobial efficiency. Nanoparticles have less inhibition effect on *S. aureus* ATCC 29737 than the polymers in free soluble form since nanoparticles have less positive charge available to bind to the negative bacterial cell wall (Sadeghi et al., 2008). Conversely, another research reported that chitosan nanoparticles exhibit higher antibacterial activity against than chitosan on account of the special character of the nanoparticles (Qi et al., 2004) (Table 2), likely the nanoparticle's larger surface area and higher affinity with bacteria cells, which yields a quantum-size effect.

The antimicrobial activity of chitosan in solid state is also affected by pH. As a pH is below the pKa, surface molecules of solid chitosan interact with circumstance similarly to soluble chitosan. In contrast, because molecules of solid chitosan tightly contact each other, Mw of chitosan is a negligible concern. As pH is above pKa, the inhibitory effect is exerted in another mechanism that will be discussed in the following section. Hence, solid chitosan and its derivatives can display antimicrobial activity over broader scale of pH value than soluble state.

Furthermore, the inhibitory activity depends on different factors, including solid surface characteristic and the morphology of the solid chitosan. Principle among these factors is the hydrophobic/hydrophilic characteristic of the surface to take effect. Owning more hydrophobic property, oleoyl-CMs showed stronger inhibitory effect on the tested bacterium than CMs. Meanwhile, by altering the hydrophilicity of surface, CMs with higher DD (sample A) cause higher inhibitory ratio than CMs with lower DD (Kong et al., 2008a). The zeta potential is another major surface characteristic, which is a good indicator of the available surface charges on the nanoparticle. The positivity of the chitosan in zeta potential relates to the portion of the chitosan that has been protonated and disassociated in water. Nanopaticles produced by N-trimethyl chitosan, with the highest zeta potential among the utilized polymers, showed the highest antibacterial inhibition against *S. aureus* (Sadeghi et al., 2008).

Morphology of solid chitosan and its derivatives involving particle size, membrane and fiber thickness lead to differing results. For example, in a study that examined the influence of particle size and shape of powdered chitosan membrane on *S. aureus*, it was found that decreasing particle size improved antibacterial activity. Powdered chitosan membrane in the range 74–500 μm looked like a flake or board, whereas in the range 37–63 μm, it looked like a sphere. The antibacterial activity of the powdered chitosan membrane depended on shape as well as specific surface area (Takahashia et al., 2008).

Table 2
MIC (μg/mL) and MBC (μg/mL) of chitosan solution, chitosan nanoparticles, and copper-loaded nanoparticles suspension at pH5.0 against various microorganism in 0.25% acetic acid.[a] Modified from Qi et al., 2004.

Bacteria	Chitosan		CNP		CNP–Cu		Doxycycline		Control	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli K88	8	64	1/16	1	1/32	1	1	4	2500	2500
E. coli ATCC 25922	8	64	1/32	2	1/32	1	2	16	2500	2500
S. choleraesuis ATCC 50020	16	32	1/16	2	1/32	1	4	32	2500	2500
S. typhimurium ATCC 50013	16	64	1/8	4	1/16	2	2	32	2500	2500
S. aureus ATCC 25923	8	32	1/8	4	1/16	2	1/4	8	2500	2500

[a] Chitosan = chitosan solution; CNP = chitosan nanoparticles suspension; CNP-Cu = copper-loaded chitosan nanoparticle suspension; doxycycline = the solution of doxycycline in 0.25% acetic acid; control = the blank tube treated just with broth and 0.25% acetic acid.

2.4. Environmental factors

2.4.1. pH

The antimicrobial activity for chitosan is pH dependent. Not just because chitosan is only soluble in an acidic environment, and the molecule becomes polycationic as pH below the molecule's pKa (6.3–6.5) (Lim and Hudson, 2004).

It has been reported that chitosan displayed antibacterial activity only in an acid environment (Helander et al., 2001), as is not proven to be strictly correct. Chitosan definitely shows stronger inhibitory effect at lower pHs, with inhibitory activity weakening with increasing pH (Kong et al., 2008b). The failure of chitosan to remain bactericidal at pH 7 may be due to the presence of a large majority of positively uncharged amino groups as well as poor solubility of chitosan (Aiedeh and Taha, 2001; Papineau et al., 1991; Sudarshan et al., 1992). However, chitosan and its derivatives completely lose their antimicrobial activities under neutral condition as reported by some workers may not be totally correct. A novel approach of antibacterial research, chitosan microsphere (CM) in solid dispersing system, showed that CM sample with DD of 62.6% exerted inhibitory effect uniquely among the three DD (97.5, 83.5, 62.6%) under neutral condition (Kong et al., 2008b). The CM samples in this experiment retained the properties of native chitosan without alteration. Another research observed that antibacterial activity of the N-alkylated chitosan derivatives (DS 30–40%) against *E. coli* increased as the pH increased from 5.0 and reached a maximum around the pH of 7.0–7.5 (Yang et al., 2005). These results also verify that positive charge on the amino groups is not the sole factor resulting in antimicrobial activities.

In contrast, virtually nothing is known about the antimicrobial activity of chitosan was available under alkaline conditions.

2.4.2. Ionic strength

Alternation of the ionic strength in a medium may disturb the inhibitory activity of chitosan (Chung et al., 2003; Raafat and Sahl, 2009), probably caused through two mechanisms. First, increase of metal ions, especially divalent ions, could attenuate the effective chelating capacity of chitosan. With the addition of 0.05 mol/L magnesium ions into a medium, the inhibitory ratio of chitosan samples decreased badly and resulted in abrogated antibacterial activity (Kong et al., 2008a). In another study, 10 and 25 mM concentrations of divalent cations reduced the antibacterial activity of shrimp chitosan against *E. coli* in the order of Ba^{2+}, Ca^{2+}, and Mg^{2+} (Tsai and Su, 1999). Furthermore, the addition of Zn^{2+} ions inhibited the antibacterial activity of chitosan most effectively compared with Ba^{2+}, Ca^{2+}, and Mg^{2+} ions (Chung et al., 2003). Secondly, along with polycationic chitosan, existing cations in medium may interact competitively with the negative components dominating on the cell wall of bacterium, consequently weakening the antimicrobial activity.

Addition of anion affected the antibacterial efficacy as well. In a study comparing the antibacterial activities of oleoyl-chitosan nanoparticles (OCNP) mixed with PO_4^{3-} of different mass ratio from 1:0.2–1:5, it was reported that the inhibition of *S. aureus* decreased as the concentration of phosphate groups increased (Xing et al., 2009a).

2.4.3. Temperature and time

For commercial applications, it would be practical to prepare chitosan solutions in bulk and to store them for further use. During storage, specific characteristics of chitosan, viscosity or Mw might be altered. Therefore, altered viscosity of a chitosan solution must be monitored since it may influence other functional properties of the solution. Stability of chitosan (Mw of 2025 and 1110 kDa) solutions and their antibacterial activity against gram-positive (*Listeria monocytogenes* and *S. aureus*) and gram-negative (*Salmonella enteritidis* and *E. coli*) bacteria were investigated at 4 °C and 25 °C after 15-week storage (No et al., 2006). Generally, chitosan solutions before storage showed higher antibacterial activity than those after 15-week storage. Chitosan solutions stored at 25 °C possessed parallel or weaker antibacterial activity compared with those at 4 °C.

In one study, the susceptibility of *E. coli* to chitosan increased upon increasing temperature from 4 to 37 °C (Tsai and Su, 1999), suggesting the low temperature stress was capable of changing the cell surface structure in a way that decreased the number of surface binding sites (or electronegativity) for chitosan derivatives.

3. Mode of antibacterial action

The exact mechanisms of the antibacterial activities of chitosan and its derivatives are still unknown. It is known that chitosan's antimicrobial activity is influenced by a number of factors that act in and orderly and independent fashion. The mode of antibacterial action is discussed in part of chitosan and microorganism below.

3.1. Part of chitosan

The polycationic structure of chitosan is a prerequisite for antibacterial activity. As environmental pH is below the pKa of chitosan and its derivatives, electrostatic interaction between the polycationic structure and the predominantly anionic components of the microorganisms' surface (such as Gram-negative lipopolysaccharide and cell surface proteins) plays a primary role in antibacterial activity.

The polycationic structure forms unnecessarily in acidic conditions, because the grafted groups of specific derivatives may change the pKa of chitosan and cause protonation at higher pH value (Yang et al., 2005). When the positive charge density of chitosan strengthens, the antibacterial property will increase consequently, as is the case with quaternized chitosan (Ignatova et al., 2006; Jia et al., 2001; Xie et al., 2007) and chitosan metal complex (Chen et al., 2005a,b; Wang et al., 2004). On the contrary, if the polycationic property of chitosan is deprived or reversed, the corresponding antibacterial capacity will be weakened or lost.

Besides protonation, the number of amino groups linking to C-2 on chitosan backbones is important in electrostatic interaction. Large amount of amino groups are able to enhance the antibacterial activity. Accordingly, native chitosan with higher DD shows a stronger inhibitory effect than that a molecule with a lower DD. Moreover, it has been reported that asparagine N-conjugated chitosan oligosaccharide that possesses two positively-charged sites provides strong

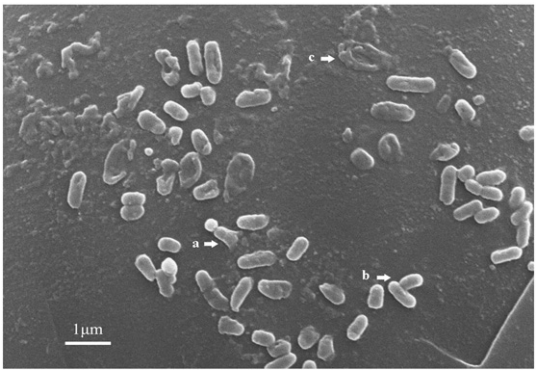

Fig. 1. SEM photographs of *E. coli* treated with CMs (97.5%) Data is based on Kong et al., 2008a.

interaction with carboxyl-negative charges on the bacteria cell wall (Jeon et al., 2001). Another attempt to increase the amount of amino groups via substituting amino by formamidine obtained a guanidinylated chitosan, which showed better antibacterial activity than chitosan (Hu et al., 2007a,b). Reversely, due to graft on the amino groups, N-carboxyethylchitosan did not possess any antibacterial activity at concentrations up to 20 mg/mL (Yancheva et al., 2007).

As environmental pH is above pKa, hydrophobic and chelating effects are responsible for antibacterial activity instead of electrostatic effect. Actually, these two effects can work beyond pH limit, however, whose efficacy are covered by the predominant electrostatic interaction under lower pH value. Nonetheless, these effects turn out to be crucial after compromise of polycationic structure. For native chitosan, lacking hydrophobicity in nature makes it exhibit inhibitory capacity chiefly by chelation of trace metal from cell wall, as pH is above pKa. In the case of chitosan derivatives, especially for those modified with lipophilic groups, the antibacterial activities are realized through both hydrophobic effect and chelation. The two effects offer a reasonable explanation for the reports that chitosan derivatives showed higher activity than native chitosan under neutral or higher pH condition (Hu et al., 2007a,b; Kong et al., 2008a; Tikhonov et al., 2006).

The different physical states and Mws of chitosan and its derivatives render distinctive modes of antibacterial action. LMw water-soluble chitosan and ultrafine nanopaticles could penetrate cell wall of bacteria and combine with DNA and inhibit synthesis of mRNA and DNA transcription (Sudarshan et al., 1992). HMw water-soluble chitosan and solid chitosan including larger size nanopaticles interact with cell surface instead and alter cell permeability resultingly (Leuba and Stossel, 1985), or form an impermeable layer around the cell, thus blocking the transport of essential solutes into the cell (Choi et al., 2001; Eaton et al., 2008). Experiments conducted with *E. coli* treated with CM and oleoyle-chitosan nanoparticles (OCNP) have revealed that the same microbial species can display significant differences in mode of action depending on the two different dimensions of chitosan particles (Kong et al., 2008a; Xing et al., 2009b). As shown in Fig. 1, the cells located on the surface of chitosan microsphere showed various states: some were intact, some were leaking intracellular substances and some had already ruptured leaving only the membrane. These results are consistent with the idea that CMs kill bacteria through an interfacial contacting inhibitory effect that occurs on the surface of the microspheres (Kong et al., 2008a). Fig. 2 displays a transmission

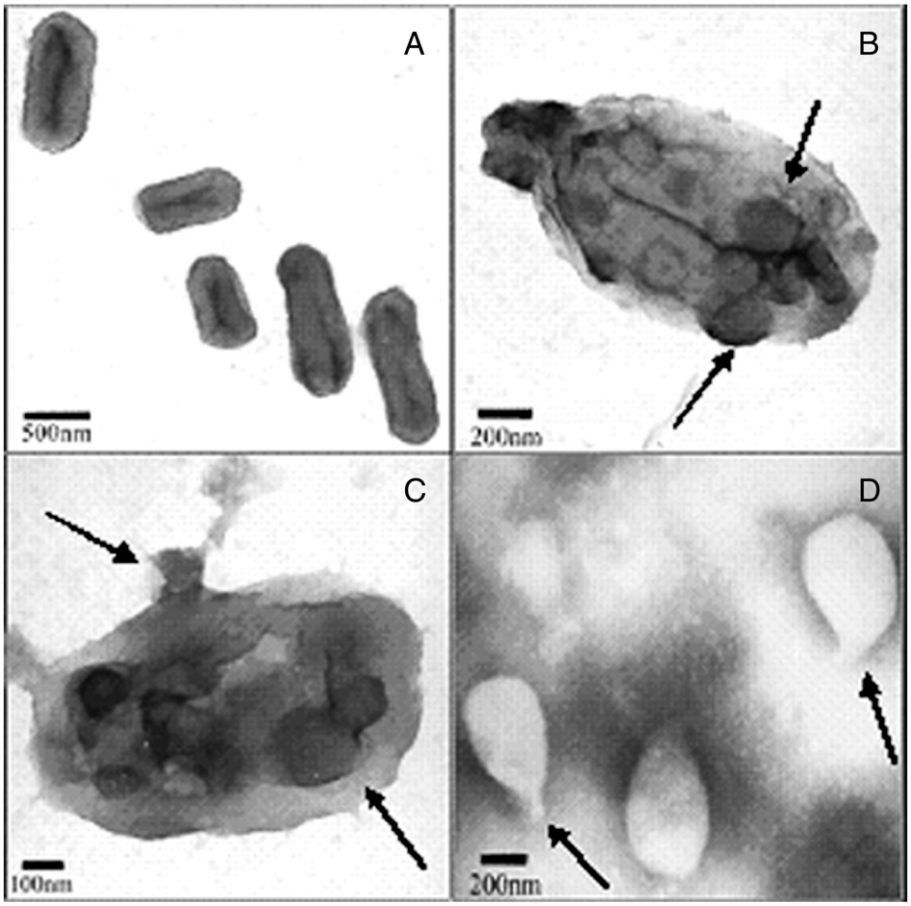

Fig. 2. TEM of *E. coli* cells treated with 300 mg/l OCNP for up to 30 min. (A) untreated *E. coli*; (B) OCNP-treated *E. coli* for 5 min; (C) OCNP-treated *E. coli* for 15 min; (D) OCNP-treated *E. coli* for 30 min. Data is based on Xing et al., 2009b.

electronic microscope (TEM) image of *E. coli* treated with 300 mg/L OCNP for up to 30 min. OCNP attached to the surface of cells after 5 min, became blurry in the reaction process while the cell surface roughened and collapsed 15 min later. The cell showed "ghost-like" appearance as well as leaking outlets suggesting empty cell envelop in Fig. 2D (Xing et al., 2009b). During the process, OCNP reacted with the cell wall components locally, as attested to the leakage of intracellular substances on certain site verified from the remaining leaking outlets. Therefore, chitosan in solid state undergoes a surface-to-surface and local reaction mode, rather than a thorough contacting mode that occurs in liquid state.

3.2. Part of microorganism

Gram-negative bacteria possess an outer membrane (OM) that contains lipopolysaccharide (LPS), which provide the bacterium with a hydrophilic surface. The lipid components and the inner core of the LPS molecules contain anionic groups (phosphate, carboxyl), which contribute to the stability of the LPS layer through electrostatic interactions with divalent cations (Fig. 3) (Helander et al., 1997). Removal of these cations by chelating agents such as ethylenediamine tetraacetic acid results in destabilization of the OM through the release of LPS molecules. The OM serves as a penetration barrier against macromolecules and hydrophobic compounds, thus Gram-negative bacteria are relatively resistant to hydrophobic antibiotics and toxic drugs. Therefore, overcoming the OM is a prerequisite for any material to exert bactericidal activity towards Gram-negative bacteria (Kong et al., 2008a).

The cell wall of Gram-positive bacteria comprises peptidoglycan (PG) and teichoic acid (TA) (Fig. 4) TA is an essential polyanionic polymer of the cell wall of Gram-positive bacteria, traversing the wall to contact with the PG layer. They can be either covalently linked to N-acetylmuramic acid of the peptidoglycan layer (wall teichoic acids) or anchored into the outer leaflet of the cytoplasmic membrane via a glycolipid (lipoteichoic acids, LTA) (Raafat et al., 2008). Poly

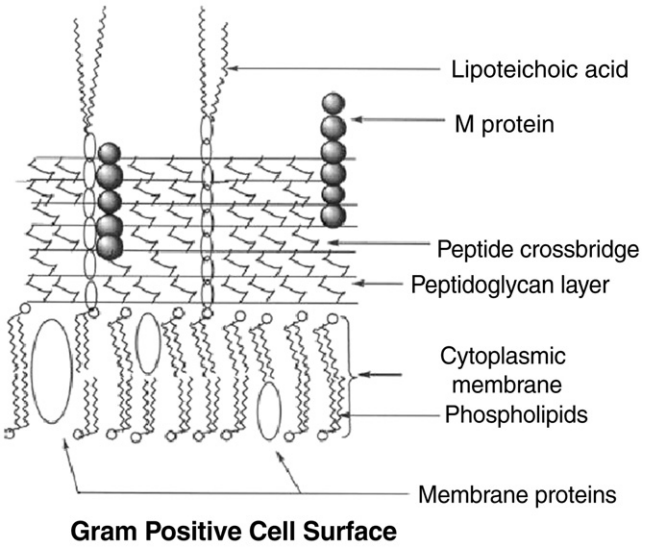

Fig. 4. Schematic view of Gram-positive bacteria cell wall.

(glycerol phosphate) anion groups make TA responsible for structural stability of cell wall. Besides, it is crucial for the function of various membrane-bound enzymes. Comparatively, TA's counterpart LPS acts similarly in the cell wall of Gram-negative bacteria.

Chitosan's antibacterial activity closely correlates with the cell surface characteristics. Bacterial surfaces are structurally complex and chemically heterogeneous, and cannot be considered as a smooth surface of a sphere. Many bacteria possess a variety of surface appendages such as pili, fimbriae or flagella, and even bacteria without these contain several types of polymers which can project from the surface, such as LPS, mycolic acids, lipoteichoic acids (LTA), capsular polysaccharides or proteins (Hancock, 1991). These polymers

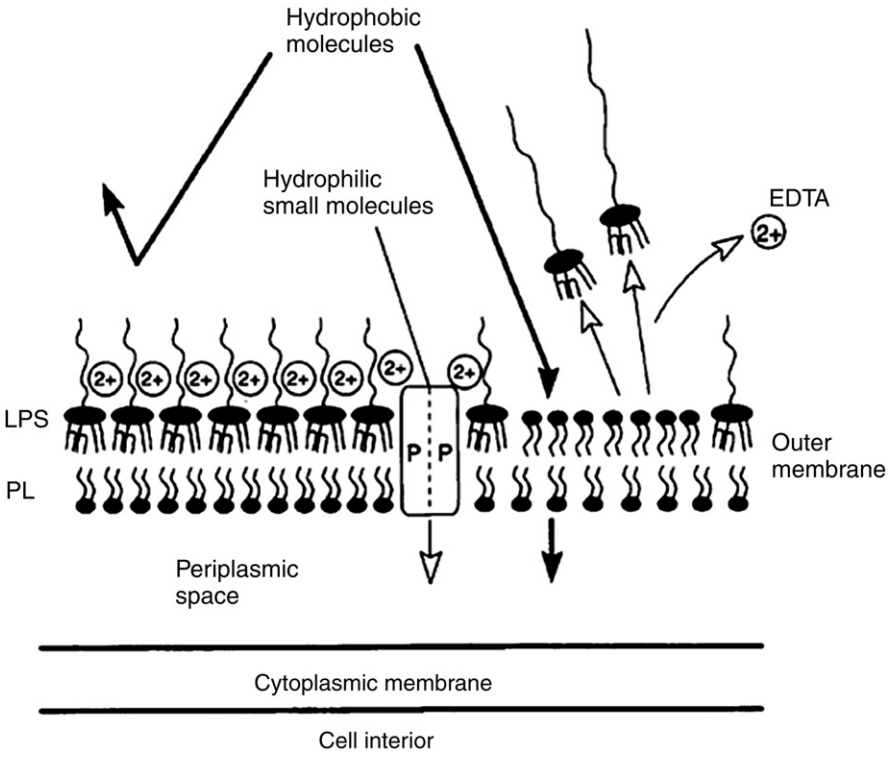

Fig. 3. Schematic view of the Gram-negative bacterial cell envelope. Data is based on Helander et al., 1997.

considerably involve in interactions with surfaces (referred to as polymer interactions) and may cause strong attachment through short-range interactions of a different nature, such as hydrogen bonding (Jucker et al., 1997). Polymers can span over relatively long distances and cause attachment even when the cells do not experience any net attraction (Jucker et al., 1998).

Polyanions on cell surface also take part in the electrostatic interactions with chitosan as well as its derivatives. The negative charge on the cell surface of the tested Gram-negative bacteria was higher than that on the tested Gram-positive bacteria, leading to more chitosan adsorbed and higher inhibitory effect against the Gram-negative bacteria (Chung et al., 2004). Cell surface hydrophobicity is another crucial factor in interactions between bacteria and surfaces, as in adhesion or floc formation (Stranda et al., 2002).

Despite the distinction between Gram-negative and Gram-positive bacterial cell walls, antibacterial modes both begin with interactions at the cell surface and compromise the cell wall or OM first. For Gram-positive bacteria, LTA could provide a molecular linkage for chitosan at the cell surface, allowing it to disturb membrane functions (Raafat et al., 2008). LPS and proteins in the Gram-negative bacteria OM are held together by electrostatic interactions with divalent cations that are required to stabilize the OM. Polycations may compete with divalent metals for binding with polyanions as pH is below pKa of chitosan and its derivatives. However, it switches to chelation as pH is above pKa. Replacement of Mg^{2+} and Ca^{2+} ions present in the cell wall will likely disrupt the integrity of the cell wall or influence the activity of degradative enzymes. The disruption of cell wall integrity has been testified by several methods. The hydrophobic probe N-phenyl-1-naphthylamine (NPN) is normally excluded from OM. When OM was damaged and functionally invalid, NPN can partition into perturbed OM, as evidenced by increased fluorescence (Je and Kim, 2006a,b; Liu et al., 2004; Rurián-Henares and Morales, 2008). Atomic force microscopy study of the antibacterial effects of chitosan revealed that, in nano-indention studies, the deflection curve for both *E. coli* and *S. aureus* treated with chitosan oligomers exhibited lower slopes than for the untreated bacteria, showing that indentation or compression of the cells had occurred due to less stiff cells after treatment. The results presumably reflect cell wall weakening, either by cell wall damage alone or accompanied by some lysis of the cell (Eaton et al., 2008).

Once the cells lose their protection of cell wall, cell membrane is unguarded to circumstance. The functions of cell membrane can be changed consequently, with membrane permeability being drastically altered (Kong et al., 2008a). Contact between chitosan and cell membrane, which is essentially a negatively charged phospholipid bilayer, may slightly change the membrane permeability. The binding also promptly neutralizes and even reverses the surface charge of the bacteria (Chen and Cooper, 2002). Further interactions may denature membrane proteins and initiate penetration into the phospholipid bilayer. The increased membrane permeability leads to destabilization of cell membrane and leakage of intracellular substances, ultimately, the death of cells.

Membrane proteins are suggested to participate in antibacterial activities. One CM-based study found conformation alterations of membrane proteins, which reflected the change the membrane constitution indirectly. The increasing fluorescence of phenylalanine (Phe) residue of treated bacterial suspension indicated that OCMs changed the structure of membrane protein and exposed the Phe residues located in the interior of membrane (Kong et al., 2008a). Whey protein was proven to have a negative effect on the antimicrobial activity of chitosan (No et al., 2007). This conclusion coincides with the reality that the use of chitosans (irrespective of Mw) will be limited to food products that possess a low protein content (Fernandes et al., 2008).

Leakage of intracellular components is a conclusive indication of damage on the bacterial cytoplasmic membrane. Evidence of leakage includes increased absorption at 260 nm (Chen and Cooper, 2002), electric conductivity of cell suspension (Kong et al., 2008a), cytoplasmic β-galactosidase release (Je and Kim, 2006a,b; Liu et al., 2004; Rurián-Henares and Morales, 2008). Moreover, microscopic images provided direct evidence of damaged cell membrane (Eaton et al., 2008; Kong et al., 2008a). The sequence of leakage caused by exposure to cationic biocides begins with LMw substances such as potassium ions and phosphate, followed by nucleotides such as DNA, RNA and other materials (Davies et al., 1968; Rye and Wiseman, 1964).

The targets on cell membrane interacting with chitosan probably are phospholipid and proteins (Chen and Cooper, 2002; Kong et al., 2008a). There are three major modes of interactions between cationic polymers and negatively charged lipids (Ostro, 1983; Seki and Tirrell, 1984). The first mode, exemplified by polylysine, seems to involve simple surface binding due to the attraction of opposite charges. This type of interaction is able to be abrogated by raising the ionic strength, and exerts little impact on the phase transition temperature of the lipid. The second type of interaction also involves charge interactions in addition to penetration of the polymer into the bilayer, which causes expansion, decrease of the phase transition temperature and alteration in the permeability of the membrane. In the third mode of interaction, polymers can completely disrupt the membrane, thus eliminate the phase transition of the lipids. In this case, formation of mixed polymer-lipid micelles or other aggregates may occur. Based on the evidence discussed above, the mechanism for chitosan is probably related to phase separation, which is similar to other cationic biocides (Chen and Cooper, 2002).

The antimicrobial activity of chitosan and its derivatives may be accounted for by the contribution of the polymers to each elementary process in the biocidal action. For example, the sequence of elementary events in the lethal action of the cationic biocide may be considered as follows: (1) adsorption onto the bacterial cell surface, (2) diffusion through the cell wall, (3) adsorption onto the cytoplasmic membrane, (4) disruption of the cytoplasmic membrane, (5) leakage of the cytoplasmic constituents, and (6) death of the cell (Ikeda and Tazuke, 1984).

So far, the mode of action is basically analyzed from physico-chemical and morphological points of view, few workers have investigated it in molecular view. Molecular methodology and considerations are able to explore the mode in more biologically intrinsic details, from fundamental metabolism and energy transit standpoint, expected to peek the nature of antimicrobial action. In one such recent study (Raafat et al., 2008), the transcriptional response pattern to chitosan revealed 166 open reading frames that showed a statistically significant (at 0.64% false discovery rate) change in expression level. Chitosan treatment reduced the bacterial growth rate, which was clearly reflected in genetic expression profiles. This transcriptional response data provides indirect evidence that chitosan treatment interferes with cellular energy metabolism. It is considered that binding of chitosan to cell wall polymers would then trigger secondary cellular effects: destabilization and subsequent disruption of bacterial membrane function occurs, albeit via unknown mechanisms, compromising the membrane barrier function and leading to leakage of cellular components without causing distinct pore formation. In addition, membrane-bound energy generation pathways are affected, probably due to impairment of the proper functional organization of the electron transport chain, thus interfering with proper oxygen reduction and forcing the cells to shift to anaerobic energy production. This might ultimately lead to dysfunction of the whole cellular apparatus.

The above discussions led to the conclusion that the mode of antimicrobial action of chitosan is not a simple mechanism but an intricate event-driven process. This process results from a sequence of quite “untargeted” molecular events taking place simultaneously and successively, rather than being confined to a certain target molecule. Nonetheless, this is just a present conclusion founding on the current research so far on the pathway to chitosan's exact antimicrobial

mechanism. Whether this conclusion is proven to be true awaits further study.

4. Antimicrobial application of chitosan and its derivatives

The ideal antimicrobial polymer should possess the following characteristics: (1) easily and inexpensively synthesized, (2) stable in long-term usage and storage at the temperature of its intended application, (3) not soluble in water for a water-disinfection application, (4) does not decompose to and/or emit toxic products, (5) should not be toxic or irritating to those who are handling it, (6) can be regenerated upon loss of activity, and (7) biocidal to a broad spectrum of pathogenic microorganisms in brief times of contact (Kenawy et al., 2007). As a natural polyaminosaccharide, chitosan possesses many of these attributes. From a biological standpoint, chitosan and its derivatives are very attractive for medical, food and textile industries, which are closely related to human safety and fitness.

4.1. Application in food industry

Chitosan has been approved as a food additive in Korea and Japan since 1995 and 1983, respectively (KFDA, 1995; Weiner, 1992). Increasing consumer demands for high-quality and microbiologically safer foods, together with longer product shelf life, are continuously forcing researchers and the industry to develop new food preservative strategies. The majority of cases associated with fresh produce are caused by *S. enteritidis*, *E. coli* O157:H7, *L. monocytogenes*, and *Campylobacter jejuni*. In particular, *E. coli* O157:H7 is an infamous foodborne pathogen that has been detected in various foods, including raw milk, cheese, undercooked meat, and spinach (Du et al., 2008). A need therefore exists to discover new, food-compatible ways to protect foods against this and other pathogens. Chitosan is mostly applied as a food additive or preservative, and as a component of packaging material, not only to retard microorganism growth in food, also to improve the quality and shelf life of food (Table 3). In this section, comparisons of different physical state of chitosan in food

Table 3
Application of antimicrobial property of chitosan.

Support (preparation method)	Application	Tested microorganism
Chitosan acetates	Food preservative[a]	*Escherichia coli* *Staphylococcus aureus*
Chitosan and its Maillard reaction products	Food preservative[a]	*Bacillus subtilis* CCRC 10258
Chitosan-hydroxy propyl methyl cellulose film	Packaging materials[a]	*Listeria monocytogenes*
Chitosan/polyethylene oxide film	Packaging materials[a]	*Escherichia coli*
Chitosan-nylon-6/Ag blended membranes	Packaging materials[a]	*Escherichia coli* *Staphylococcus aureus*
Polypropylene/chitosan/pectin films	Packaging materials[a]	Bacteria: *Clavibacter michiganensis* *Pseudomonas solanacearum* Fungi: *Fusarium oxysporum* *Verticillium albo-atrum* *Alternaria solani*
Chitosan-hydroxy propyl methyl cellulose film	Edible films and coatings[a]	*Aspergillus niger*
Chitosan	Food additive[a]	*Streptococcus*
Alginate/chitosan fibers	Wound dressing materials[b]	*Staphylococcus aureus*
Quaternised chitosan nano-fibers	Wound-healing applications[b]	*Escherichia coli* 3588 *Staphylococcus aureus* 749
Quaternized chitosan derivative/poly (vinyl pyrrolidone) fibers	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Alginate/carboxymethyl chitosan blend fibers	Wound dressing materials[b]	*Staphylococcus aureus*
Polypropylene-g-acrylic acid-g-N-isopropylacrylamide-chitosan fabric	Wound dressing materials[b]	*Pseudomonas aeruginosa* *Staphylococcus aureus*
Chitosan/cellulose blends membrane	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Chitosan-$Ca_3V_{10}O_{28}$ complex membrane	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Porous chitosan/poly(N-isopropylacrylamide) gel/polypropylene sponge	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Chitosan-gelatin sponge	Wound dressing materials[b]	*Escherichia coli* K88 *Streptococcus*
Photocrosslinkable chitosan hydrogel	Wound dressing and tissue adhesion[b]	*Escherichia coli*
Poly(vinyl alcohol)/water-soluble-chitosan hydrogels	Wound dressing materials[b]	*Escherichia coli*
Chitosan/poly(vinyl alcohol) blended hydrogel membranes	Haemodialysis[b]	*Aurococcus*
Polyacrylonitrile/chitosan/heparin	Haemodialysis[b]	*Pseudomonas aeruginosa* ATCC 10145
6-O-carboxymethylchitosan/waterborne polyurethanes semi-interpenetrating polymer network membranes	Biomaterial for blood-contracting devices[b]	*Escherichia coli*
Chitosan/heparin multilayer films	Tissue engineering[b]	*Escherichia coli*
Trimethyl chitosan and N-diethylmethyl chitosan nanoparticles loaded with insulin	Delivery system[b]	*Staphylococcus aureus* ATCC 29737
N-carboxymethylchitosan N,O-sulfate	Drugs for AIDS[b]	*HIV*-1
Water soluble carboxymethyl chitosan	Cotton fabric[c]	*Escherichia coli* *Staphylococcus aureus*
Poly(n-butyl acrylate) cores and chitosan shells core-shell particles	Cotton fabric[c]	*Staphylococcus aureus*

Chen et al., 2005a,b, 2006; Deng et al., 2007; Fan et al., 2006; Fu et al., 2005; Fujita et al., 2004; Gama Sosa et al., 1991; Gupta et al., 2007; Hayashi et al., 2007a; Huang et al., 2007; Ignatova et al., 2006, 2007; Knilla et al., 2004; Li et al., 2007; Lin et al., 2004; Ma et al., 2008; Maher et al., 2008; Moller 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; Zivanovic et al., 2007.

[a] Food industry.
[b] Medical industry.
[c] Textile industry.

application were discussed, and part of innovative processing methods were introduced as well.

Low water solubility forces chitosan to be dissolved in dilute acid solution, such as acetic or lactic acid solution, in many food applications (No et al., 2007). This acidic ambience can adversely affect chitosan molecules via hydrolysis and chain depolymerization. The development of modified water-soluble derivative is an efficient approach in practice and the chitosan structure is chemically more stable compared with the acidic solution. It was observed that, chitosan acetate inhibited the growth of two main waterborne food pathogens, *E. coli* and *S. aureus* (Li et al., 2007). Physiological effects of Maillard reaction in food system are growing to be point of interest, owing to the reducing sugar is one of the most commonly prevalent chemical compounds that exist during food processing and preservation. It was reported that addition of 0.05 g/100 mL chitosan (in 0.5 mL/100 mL acetic acid) to a fresh noodle formulation resulted in an extension of its shelf-life to another 6 days when stored at 4 °C. However, addition of Maillard reaction products prepared from chitosan and xylose resulted in the longer shelf-life lasting 14 days when stored at 4 °C (Huang et al., 2007).

Compared with a solution, film based food preservative materials are safer and more widely applied presently, especially as food package. Various kinds of chitosan-based packaging films modified with new polymeric material have been developed. The process endows these cooperating films with antimicrobial property as well as advantageous mechanical characteristics (Table 3) (Ma et al., 2008; Maher et al., 2008; Moller et al., 2004; Zivanovic et al., 2007). As well, instead of polyethylene or polypropylene petrochemical materials those are inedible or not made from renewable natural resources, demands for biodegradable and environmentally-friendly packaging have spurred interest in biodegradable, polymer-based edible packaging films. Chitosan is ideally positioned in this regard.

Antimicrobial properties of edible antimicrobial films prepared from chitosan and other edible food ingredients, for example chitosan with essential oils, chitosan with nisin, chitosan-glucomannan-nisin blends, chitosan-starch blends (Du et al., 2008). A study of the antimicrobial properties of chitosan films containing anise, basil, coriander and oregano plant essential oils reported similar results to pure oils (Zivanovic et al., 2005). Some of these films also inactivated *L. monocytogenes* and *E. coli* O157:H7 pathogens on the surface of meat products. Relating Studies on chitosan based antimicrobial film have been systematically and comprehensively discussed (Dutta et al., 2009).

Efforts have exploited chitosan in innovative and convenient manners to broaden its application. In one study conducted in Japan, researchers confirmed the mechanical efficacy of chewing chitosan-containing gum to suppress the growth of oral bacteria compared to a mouth rinse, and demonstrated the increased salivary secretion due to chewing chitosan-containing gum (Hayashi et al., 2007a). Recent studies have demonstrated that chewing chitosan-containing gum effectively inhibited the growth of cariogenic bacteria (total bacteria, total *Streptococci*, *mutans streptococci*) in saliva (Hayashi et al., 2007b).

4.2. Application in medical industry

In the area of health care and hygienic applications, biocidal polymers may be incorporated into fibers, membrane, or hydrogel, and used for contact disinfectants in many biomedical applications, including wound dressing, orthopaedic tissue engineering, drug delivery carrier and haemodialysis.

Generally, an ideal wound dressing material must be capable of absorbing the exuded liquid from the wounded area and should permit water evaporation at a certain rate and allow no microbial transport (Yang et al., 2004). As a key parameter regarding wound dressing, the antimicrobial property assessment is necessary for evaluating the eligibility and capability of the candidate. Polysaccharides, e.g. chitosan, owning hydrogel-forming properties have been considered to be advantageous in their application as a wound dressing materials (Chen et al., 2005a,b). Chitosan-based materials have received much attention in this regard.

Typically, there are four forms in which chitosan provides antimicrobial effect to wound dressing materials: fiber, membrane, sponge and hydrogel (Table 3). The different approaches count on particular physicochemical characteristics of chitosan, which impart talent on specific displaying form.

Majority of antimicrobial products perform their talent in fabric form. Micro- and nanofiber materials are suitable for preparing wound dressings. Among these, electrospinning is a favorable technique for producing continuous polymer fibers with diameters down to nano-scale range (Deitzel et al., 2001). Because of unique properties such as high surface-to-volume ratio, high porosity and diameters in the nano-scale, electrospun mats made from ultrafine polymer fibers have been drawing great attention. One study reported found the crosslinked QCh/PVP (quaternized chitosan/poly vinyl pyrrolidone) electrospun materials were efficient in inhibiting growth of Gram-positive bacteria and Gram-negative bacteria (Ignatova et al., 2007). While, in their previous work (Ignatova et al., 2006), the antibacterial activity of cross-linked electrospun QCh/PVA (poly vinyl alcohol) mats made of quaternized chitosan derivative against *S. aureus* was observed to be bactericidal rather than bacteriostatic. PVP and PVA are both non-toxic, biocompatibility, high hydrophilicity, possess good complexation properties and have good film-forming ability, which are crucial for wound-healing materials. In another work, alginate/CM-chitosan blend fibers were prepared by spinning. The introduction of CM-chitosan not only improved water-retention properties of the blend fiber compared to that of pure alginate fiber, and also endowed the fiber with good antibacterial activity against *S. aureus* (Fan et al., 2006).

Membrane material also appeared especially advantageous. Blends of chitosan and cellulose have been prepared by casting films from trifluoroacetic acid. Besides the lower water vapor transpiration rate, which prevented excessive dehydration of the wound, chitosan/cellulose blend membrane also exhibited effective antimicrobial capability against *E. coli* and *S. aureus* (Wu et al., 2004). Another novel chitosan-$Ca_3V_{10}O_{28}$ complex membrane with sustained antimicrobial capability was prepared by self-assembly of $V_{10}O_{28}^{6-}$ and chitosan, where Ca^{2+} was used as the linker. Owing to the synergistic effect of both components, the chitosan-$CaV_{10}O_{28}$ complex displayed much higher antimicrobial activity than the individual component against *S. aureus* and *E. coli* (Chen et al., 2006).

Immobilized onto poly(N-isopropylacrylamide) gel/polypropylene nonwoven composites surface using the cross-linking agent, glutaraldehyde, chitosan hydrogels displayed antibacterial ability to *E. coli* and *S. aureus* while untreated nonwoven, as a control, did not give any change on bacteria number. Meanwhile, the product showed easily stripped-off property without damaging the new regenerated tissue as it should be removed from the wound (Chen et al., 2005a,b).

Referring to surgical and pharmaceutical material introduced into human body, tissue engineering and drug release system for instance, risks from complications arising from microorganism infections are ubiquitous clinical problem. It is apparent that, once the introduced materials are infected, high morbidity and mortality rate can be expected. Therefore, several efforts have focused on the development of bacterial-resistant prosthetic counterparts through binding of an antibiotic to the materials. For example, chitosan hydrogelcoated grafts, crosslinked upon ultraviolet light irradiation, exhibited a resistance against *E. coli* in vitro and in vivo (Fujita et al., 2004). The photocrosslinkable chitosan hydrogel directly acted as antibacterial biomaterial on a Dacron graft, and at least chitosan hydrogel was effective to inhibit the local infection. A branch-type of galactosylated chitosan was promising as a novel glycoconjugated macromolecule for a specific liver targeting drug delivery system. Galactosylated chitosan (gal-chit and gal-lys-chit) films with hydrophilic galactosyl

groups might be a potential biomaterial with antimicrobial ability (Mi et al., 2006).

A study that investigated *N*-carboxymethylchitosan *N*,*O*-sulfate, a heparin-like polysaccharide derived from *N*-carboxymethyl chitosan by a random sulfation reaction, demonstrated the ability of the polysaccharide to inhibit the replication of human immunodeficiency virus-1 and viral binding with CD4 (Gama Sosa et al., 1991). The selective sulfation at *O*-2 and/or *O*-3 may generate potent antiretroviral agents that display a much higher inhibitory effect on the infection of AIDS virus than that by the known 6-*O*-sulfated derivative (6-sulfate) (Nishimura et al., 1998).

4.3. Application in textile industry

Antimicrobial treatment is increasingly becoming a standard finish for some textile products such as for medical, institutional and hygienic uses. Recently, it has became popular in sportswear, women's wear, and aesthetic clothing to impart anti-odor or biostatic properties (Kenawy et al., 2007).

Natural textiles such as those made from cellulose and protein fibers are often considered to be more vulnerable to microbial attack than man-made fibers in light of their hydrophilic porous structure and moisture transport characteristics. Thus, the use of antibacterial agents to prevent or retard the growth of bacteria is becoming a standard finishing for textile goods. There is, however, increasing public concern over the possible effects of antibacterial finishing on environmental and biological systems since many antibacterial agents are toxic chemicals, lack of efficiency and durability (Ye et al., 2005). Accordingly, an ideal textile antibacterial finishing should be safe and environmentally benign besides killing undesirable micro-organisms. Chitosan, as nontoxic, biodegradable and biocompatible natural polymer, as well as having antimicrobial activity, is an ideal candidate material.

Cotton fabric treated with water-soluble carboxymethyl chitosan showed good antimicrobial activity against *E. coli* and *S. aureus* at 0.1% concentration as well as improved wrinkle recovery (Gupta and Haile, 2007).

Chitosan-based core-shell particle, with chitosan as the shell and poly(n-butyl acrylate) (PBA) as the core, has been designed as a novel antibacterial coating for textiles (Ye et al., 2005). Cotton treated with PBA-chitosan particles demonstrates an excellent antibacterial activity against *S. aureus* with bacterial reductions more than 99%.

5. Perspectives and future

Over the last decades, considerable interest and attention have been focused on chitosan ascribing from its potential and advantages as antimicrobial agent. Investigations on its antimicrobial property originated from conventional morphology observation to micron and submicron inner structural metabolism study. The methods and technologies used to evaluate the phenomena and results have gone beyond sole biological conception, but yet incorporate a combination of disciplines involving chemistry, physics, informatics, nanotechnology and genetic engineering. The focus will persist and more thorough comprehension about the mode of action, which is also beneficial for the exploitation of new generation of antimicrobial agents and for the development of new biomedicine.

Looking back through the past research, some drawbacks have become apparent, which should be the focus of future work. One of those is the various results reported by researchers even under identical conditions. The assay conditions have not been standardized and it is difficult to make comparison from one study with another. Obviously, if the issue were not solved, more reduplicate and nonsense job would be done as well as tremendous amount of time and resources would be wasted in vain. In this regard, a set of uniform and standard measurement is requisite to be established or perfect the existing criterion further, such as the Clinical and Laboratory Standards Institute (CLSI) methods for dilution antimicrobial susceptibility test from bacteria that grow aerobically (former National Committee for Clinical Laboratory Standards, NCCLS) (CLSI, 2006).

In the case of antimicrobial mode of action, future work should aim at clarifying the molecular details of the underlying mechanisms and their relevance to the antimicrobial activity of chitosan. Moreover, further investigations in this area, in particular with regard to bacterial resistance mechanisms against this compound, are warranted. In addition, participation and collaboration of research institutes, industry, and government regulatory agencies will be the key for the success of antimicrobial mechanism.

Acknowledgments

This work was supported by grants from NSFC (30770582), ISTCP (2006DFA33150) and the Shandong Science Foundation (Y2006C110), P. R. China.

References

Aiedeh, K., Taha, M.O., 2001. Synthesis of iron-crosslinked chitosan succinate and iron-crosslinked hydroxamated chitosan succinate and their in vitro evaluation as potential matrix materials for oral theophylline sustained-release beads. European Journal of Pharmaceutical Sciences 13, 159–168.

Allan, C.R., Hardwiger, L.A., 1979. The fungicidal effect of chitosan on fungi of varying cell wall composition. Experimental Mycology 3, 285–287.

Arvanitoyannis, I.S., 1999. Totally and partially biodegradable polymer blends based on natural and synthetic macromolecules: preparation, physical properties, and potential as food packaging materials. Journal of Macromolecular Science-Reviews in Macromolecular Chemistry and Physics C 39, 205–271.

Arvanitoyannis, I.S., Nakayama, A., Aiba, S., 1998. Chitosan and gelatin based edible films: state diagrams, mechanical and permeation properties. Carbohydrate Polymers 37, 371–382.

Bayer, M.E., Sloyer, J.L., 1990. The electrophoretic mobility of Gram-negative and Gram-positive bacteria: an electrokinetic analysis. Journal of General Microbiology 136, 867–874.

Chen, Y.L., Chou, C.C., 2005. Factors affecting the susceptibility of *Staphylococcus aureus* CCRC 12657 to water soluble lactose chitosan derivative. Food Microbiology 2005 (22), 29–35.

Chen, C.Z.S., Cooper, S.L., 2002. Interactions between dendrimer biocides and bacterial membranes. Biomaterials 23, 3359–3368.

Chen, K.S., Ku, Y.A., Lee, C.H., Lin, H.R., Lin, F.H., Chen, T.M., 2005a. Immobilization of chitosan gel with cross-linking reagent on PNIPAAm/gel/PP non-woven composite surface. Material Sciences & Engineering-C 25, 472–478.

Chen, S.P., Wu, G.Z., Zeng, H.Y., 2005b. Preparation of high antimicrobial activity thiourea chitosan-Ag + complex. Carbohydrate Polymer 60, 33–38.

Chen, S.P., Wu, G.Z., Long, D.W., Liu, Y.D., 2006. Preparation, characterization and antibacterial activity of chitosan-Ca3V10O28 complex membrane. Carbohydrate Polymers 64, 92–97.

Choi, B.K., Kim, K.Y., Yoo, Y.J., Oh, S.J., Choi, J.H., Kim, C.Y., 2001. In vitro antimicrobial activity of a chitooligosaccharide mixture against *Actinobacillus actinomycetemcomitans* and *Streptococcus mutans*. International Journal of Antimicrobial Agents 18, 553–557.

Chung, Y.C., Wang, H.L., Chen, Y.M., Li, S.L., 2003. Effect of abiotic factors on the antibacterial activity of chitosan against waterborne pathogens. Bioresource Technology 88, 179–184.

Chung, Y.C., Su, Y.P., Chen, C.C., Jia, G., Wang, H.L., Wu, J.C.G., Lin, J.G., 2004. Relationship between antibacterial activity of chitosans and surface characteristics of cell wall. Acta Pharmacologica Sinica 25, 932–936.

Chung, Y.C., Kuo, C.L., Chen, C.C., 2005. Preparation and important functional properties of water-soluble chitosan produced through Maillard reaction. Bioresource Technology 96, 1473–1482.

CLSI, 2006. Clinical and Laboratory Standards Institute Quality Manual, 3 rd Ed. CLSI, USA.

Davies, A., Bentley, M., Field, B.S., 1968. Comparison of action of vantocil cetrimide and chlorhexidine on *Escherichia coli* and its spheroplasts and protoplasts of gram positive bacteria. The Journal of Applied Bacteriology 31, 448–452.

Deitzel, J.M., Kleinmeyer, J., Harris, D., Beck, T.N.C., 2001. The effect of processing variables on the morphology of electrospun nanofibers and textiles. Polymer 42, 261–272.

Deng, C.M., He, L.Z., Zhao, M., Yang, D., Liu, Y., 2007. Biological properties of the chitosan-gelatin sponge wound dressing. Carbohydrate Polymers 69, 583–589.

Du, W.X., Olsen, C.W., Avena-bustillos, R.J., Mchugh, T.H., Levin, C.E., Friedman, M., 2008. Storage stability and antibacterial activity against *Escherichia coli* O157: H7 of carvacrol in edible apple films made by two different casting methods. Journal of Agricultural and Food Chemistry 56, 3082–3088.

Dutta, P.K., Dutta, J., Tripathi, V.S., 2004. Chitin and chitosan: chemistry, properties and applications. Journal of Scientific and Industrial Research 63, 20–31.

Dutta, P.K., Tripathi, S., Mehrotra, G.K., Dutta, J., 2009. Perspectives for chitosan based antimicrobial films in food applications. Food Chemistry 114, 1173–1182.

Eaton, P., Fernandes, J.C., Pereira, E., Pintado, M.E., Malcata, F.X., 2008. Atomic force microscopy study of the antibacterial effects of chitosans on *Escherichia coli* and *Staphylococcus aureus*. Ultramicroscopy 108, 1128–1134.
Fan, L.H., Du, Y.M., Zhang, B.Z., Yang, J.H., Zhou, J.P., Kennedy, J.F., 2006. Preparation and properties of alginate/carboxymethyl chitosan blend fibers. Carbohydrate Polymers 65, 447–452.
Fernandes, J.C., Tavaria, F.K., Soares, J.C., Ramos, O.S., João Monteiro, M., Pintado, M.E., Xavier Malcata, F., 2008. Antimicrobial effects of chitosans and chitooligosaccharides, upon *Staphylococcus aureus* and *Escherichia coli*, in food model systems. Food Microbiology 25, 922–928.
Franklin, T.J., Snow, G.A., 1981. Biochemistry of Antimicrobial Action, 3rd ed. Chapman and Hall, London, p. 175.
Fu, J.H., Ji, J., Yuan, W.Y., Shen, J.C., 2005. Construction and enzymatic degradation of multilayered poly-l-lysine/DNA films. Biomaterials 26, 6684–6692.
Fujita, M., Kinoshita, M., Ishihara, M., Kanatani, Y., Morimoto, Y., Simizu, M., Ishizuka, T., Saito, Y., Yura, H., Matsui, T., Takase, B., Hattori, H., Kikuchi, M., Maehara, T., 2004. Inhibition of vascular prosthetic graft infection using a photocrosslinkable chitosan hydrogel. The Journal of Surgical Research 121, 135–140.
Gama Sosa, M., Fazely, F., Koch, J.A., Vercellotti, S.V., Ruprecht, R.M., 1991. N-Carboxymethylchitosan-N, O-sulfate as an anti-HIV-1 agent. Biochemical and Biophysical Research Communications 174, 489–496.
Gupta, D., Haile, A., 2007. Multifunctional properties of cotton fabric treated with chitosan and carboxymethyl chitosan. Carbohydrate Polymers 69, 164–171.
Hancock, S., 1991. In: Mozes, N., Handley, P.S., Busscher, H.J., Rouxhet, P.G. (Eds.), Cell surface analysis. VCH Publishers, Weinheim, p. 23.
Haque, T., Chen, H., Ouyang, W., Martoni, C., Lawuyi, B., Urbanska, A.M., Prakash, S., 2005. Superior cell delivery features of poly(ethylene glycol) incorporated alginate, chitosan, and poly-L-lysine microcapsules. Molecular Pharmaceutics 2, 29–36.
Hayashi, Y., Ohara, N., Ganno, T., Ishizaki, H., Yanagiguchi, K., 2007a. Chitosan-containing gum chewing accelerates antibacterial effect with an increase in salivary secretion. Journal of Dentistry 35, 871–874.
Hayashi, Y., Ohara, N., Ganno, T., Yamaguchi, K., Ishizaki, T., Nakamura, T., Sato, M., 2007b. Chewing chitosan-containing gum effectively inhibits the growth of cariogenic bacteria. Archives of Oral Biology 52, 290–294.
Helander, I.M., Wright, A.V., Mattila-Sandholm, T.M., 1997. Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria. Trends in Food Science and Technology 8, 146–150.
Helander, I.M., Nurmiaho-Lassila, E.L., Ahvenainen, R., Rhoades, J., Roller, S., 2001. Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. International Journal of Food Microbiology 71, 235–244.
Hernandez-Lauzardo, A.N., Bautista-Banos, S., Velazquez-del Valle, M.G., Mendez-Montealvo, M.G., Sanchez-Rivera, M.M., Bello-Perez, L.A., 2008. Antifungal effects of chitosan with different molecular weights on in vitro development of *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. Carbohydrate Polymers 73, 541–547.
Hirano, S., Nagao, N., 1989. Effects of chitosan, pectic acid, lysozyme, andchitinase on the growth of several phytopathogens. Agricultural and Biological Chemistry 53, 3065–3066.
Hu, Y., Du, Y.M., Yang, J.H., Kennedy, J.F., Wang, X.H., Wang, L.S., 2007a. Synthesis, characterization and antibacterial activity of guanidinylated chitosan. Carbohydrate Polymers 67, 66–72.
Hu, Y., Du, Y.M., Yang, J.H., Tang, Y.F., Li, J., Wang, X.Y., 2007b. Self-aggregation and antibacterial activity of N-acylated chitosan. Polymer 48, 3098–3106.
Huang, R.H., Du, Y.M., Zheng, L.S., Liu, H., Fan, L.H., 2004. A new approach to chemically modified chitosan sulfates and study of their influences on the inhibition of *Escherichia coli* and *Staphylococcus aureus* growth. Reactive and Functional Polymers 59, 41–51.
Huang, J.R., Huang, C.Y., Huang, Y.W., Chen, R.H., 2007. Shelf-life of fresh noodles as affected by chitosan and its Maillard reaction products. LWT 40, 1287–1291.
Ignatova, M., Starbova, K., Manolova, N., Manolova, N., Rashkov, I., 2006. Electrospun nano-fibre mats with antibacterial properties from quaternised chitosan and poly (vinyl alcohol). Carbohydrate Research 341, 2098–2107.
Ignatova, M., Manolova, N., Rashkov, I., 2007. Novel antibacterial fibers of quaternized chitosan and poly(vinyl pyrrolidone) prepared by electrospinning. European Polymer Journal 43, 1112–1122.
Ikeda, T., Tazuke, S., 1984. Biologically-active polycations.4. Synthesis and antimicrobial activity of poly(Trialkylvinylbenzylammonium chloride). Makromolekulare Chemie-Macromolecular Chemistry and Physics 185, 869–876.
Je, J.Y., Kim, S.K., 2006a. Antimicrobial action of novel chitin derivative. Biochimica et Biophysica Acta 1760, 104–109.
Je, J.Y., Kim, S.K., 2006b. Chitosan derivatives killed bacteria by disrupting the outer and inner membrane. Journal of Agricultural and Food Chemistry 54, 6629–6633.
Jeon, Y.J., Park, P.J., Kim, S.K., 2001. Antimicrobial effect of chitooligosaccharides produced by bioreactor. Carbohydrate Polymers 44, 71–76.
Jia, Z.S., Shen, D.F., Xu, W.L., 2001. Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. Carbohydrate Research 333, 1–6.
Jucker, B.A., Harms, H., Hug, S.J., Zehnder, A.J.B., 1997. Adsorption of bacterial surface polysaccharides on mineral oxides is mediated by hydrogen bonds. Colloids and Surfaces, B: Biointerfaces 9, 331–343.
Jucker, B.A., Zehnder, A.J.B., Harms, H., 1998. Quantification of polymer interactions in bacterial adhesion. Environmental Science & Technology 32, 2909–2915.
Kenawy, E.R., Worley, S.D., Broughton, R., 2007. The chemistry and applications of antimicrobial polymers: a state-of-the-art review. Biomacromolecules 2007 (8), 1359–1384.
Kendra, D.F., Hadwiser, L.A., 1984. Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation in *Pisum sativum*. Experimental Mycology 8, 276–281.
KFDA, 1995. Food additives code. Korea Food and Drug Administration, Seoul, p. 449.
Kim, H.J., Chen, F., Wang, X., Rajapakse, N.C., 2005. Effect of chitosan on the biological properties of sweet basil (*Ocimum basilicum* L.). Journal of Agriculture and Food Chemistry 53, 3696–3701.
Knilla, C.J., Kennedy, J.F., Mistry, J., Miraftab, M., Smart, G., Groocock, M.R., Williams, H.J., 2004. Alginate fibres modified with unhydrolysed and hydrolysed chitosans for wound dressings. Carbohydrate Polymers 55, 65–76.
Kong, M., Chen, X.G., Liu, C.S., Liu, C.G., Meng, X.H., Yu, L.J., 2008a. Antibacterial mechanism of chitosan microspheres in a solid dispersing system against *E. coli*. Colloids and Surfaces. B, Biointerfaces 65, 197–202.
Kong, M., Chen, X.G., Liu, C.S., Yu, L.J., Ji, Q.X., Xue, Y.P., Cha, D.S., Park, H.J., 2008b. Preparation and antibacterial activity of chitosan microspheres in a solid dispersing system. Frontiers of Materials Science in China 2, 214–220.
Kurita, K., 1998. Chemistry and application of chitin and chitosan. Polymer Degradation and Stability 59, 117–120.
Kurita, K., Kamiya, M., Nishimura, S.I., 1991. Solubilization of a rigid polysaccharide: controlled partial n-acetylation of chitosan to develop solubility. Carbohydrate Polymers 16, 83–92.
Leuba, S., Stossel, P., 1985. Chitin in Nature and Technology. Plenum Press, New York, p. 217.
Li, Y., Chen, X.G., Liu, N., Liu, C.S., Liu, C.G., Meng, X.H., Yu, L.J., Kenendy, J.F., 2007. Physicochemical characterization and antibacterial property of chitosan acetates. Carbohydrate Polymers 67, 227–232.
Lim, S.H., Hudson, S.M., 2004. Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group. Carbohydrate Research 339, 313–319.
Lin, W.C., Liu, T.Y., Yang, M.C., 2004. Hemocompatibility of polyacrylonitrile dialysis membrane immobilized with chitosan and heparin conjugate. Biomaterials 25, 1947–1957.
Liu, H., Du, Y.M., Wang, X.H., Sun, L.P., 2004. Chitosan kills bacteria through cell membrane damage. International Journal of Food Microbiology 95, 147–155.
Ma, Y.L., Zhou, T., Zhao, C.S., 2008. Preparation of chitosan-nylon-6 blended membranes containing silver ions as antibacterial materials. Carbohydrate Research 343, 230–237.
Maher, Z., Elsabee, E.S., Abdou, K.S.A., Nagy, M.E., 2008. Surface modification of polypropylene films by chitosan and chitosan/pectin multilayer. Carbohydrate Polymers 71, 187–195.
Mi, F.L., Yu, S.H., Peng, C.K., Sung, H.W., Shyu, S.S., Liang, H.F., Huang, M.F., Wang, C.C., 2006. Synthesis and characterization of a novel glycoconjugated macromolecule. Polymer 47, 4348–4358.
Moller, H., Grelier, S., Pardon, P., Coma, V., 2004. Antimicrobial and physicochemical properties of chitosan-HPMC-based films. Journal of Agricultural and Food Chemistry 52, 6585–6591.
Nishimura, S., Kai, H., Shinada, K., Yoshida, T., Tokura, S., Kurita, K., 1998. Regioselective syntheses of sulfated polysaccharides: specific anti-HIV-1 activity of novel chitin sulfates. Carbohydrate Research 306, 427–433.
No, H.K., Park, N.Y., Lee, S.H., Meyers, S.P., 2002. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. International Journal of Food Microbiology 74, 65–72.
No, H.K., Kim, S.H., Lee, S.H., Park, N.Y., Prinyawiwatkul, W., 2006. Stability and antibacterial activity of chitosan solutions affected by storage temperature and time. Carbohydrate Polymers 65, 174–178.
No, H.K., Meyers, S.P., Prinyawiwatkul, W., Xu, Z., 2007. Applications of chitosan for improvement of quality and shelf life of food: a review. Journal of Food Science 72, 87–100.
Ostro, M.J., 1983. In: Ostro, M.J. (Ed.), Liposomes. New York, Marcel Dekker.
Papineau, A.M., Hoover, D.G., Knorr, D., Farkas, D.F., 1991. Antimicrobial effect of water-soluble chitosans with high hydrostatic pressure. Food Biotechnology 5, 45–57.
Phaechamud, T., 2008. Hydrophobically modified chitosans and their pharmaceutical applications. Journal of Pharmaceutical Science and Technology 1, 2–9.
Qi, L.F., Xu, Z.R., Jiang, X., Hu, C.H., Zou, X.F., 2004. Preparation and antibacterial activity of chitosan nanoparticles. Carbohydrate Research 339, 2693–2700.
Raafat, D., Sahl, H.G., 2009. Chitosan and its antimicrobial potential – a critical literature survey. Microbial Biotechnology 2, 186–201.
Raafat, D., Bargen, K.V., Haas, A., Sahl, H.G., 2008. Insights into the mode of action of chitosan as an antibacterial compound. Applied and Environmental Microbiology 74, 3764–3773.
Rabea, E.I., Badawy, M.E.T., Stevens, C.V., Smagghe, G., Steurbaut, W., 2003. Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 4, 1457–1465.
Roberts, G.A.F., 1992. Chitin Chemistry. MacMillan Press, London, p. 350.
Roller, S., Covill, N., 1999. The antifungal properties of chitosan in laboratory media and apple juice. International Journal of Food Microbiology 47, 67–77.
Rurián-Henares, J.A., Morales, F.J., 2008. Antimicrobial activity of melanoidins against *Escherichia coli* is mediated by a membrane-damage mechanism. Journal of Agricultural and Food Chemistry 56, 2357–2362.
Rye, R.M., Wiseman, D., 1964. Release of phosphorus-32-containing compounds from micrococcus lysodeikticus treated with chlorhexidine. The Journal of Pharmacy and Pharmacology 20, 145–178.
Sadeghi, A.M.M., Dorkoosh, F.A., Avadi, M.R., Saadat, P., Rafiee-Tehrani, M., Junginger, H.E., 2008. Preparation, characterization and antibacterial activities of chitosan, N-trimethyl chitosan (TMC) and N-diethylmethyl chitosan (DEMC) nanoparticles loaded with insulin using both the ionotropic gelation and polyelectrolyte complexation methods. International Journal of Pharmaceutics 355, 299–306.
Sajomsang, W., Tantayanon, S., Tangpasuthadol, V., Daly, W.H., 2007. Synthesis of methylated chitosan containing aromatic moieties: chemoselectivity and effect on molecular weight. Carbohydrate Polymers 72, 740–750.

Sebti, I., Chillet, E., Degraeve, P., Noel, C., Petrol, E., 2007. Water sensitivity, antimicrobial, and physicochemical analyses of edible films based on HPMC and/or chitosan. Journal of Agricultural and Food Chemistry 55, 693–699.

Seki, K., Tirrell, D.A., 1984. Interactions of synthetic-polymers with cell-membranes and model membrane systems.5. ph-dependent complexation of poly(acrylic acid) derivatives with phospholipid vesicle membranes. Macromolecules 17, 1692–16928.

Stranda, S.P., Nordengenb, T., Otgaard, K., 2002. Efficiency of chitosans applied for flocculation of different bacteria. Water Research 36, 4745–4752.

Sudarshan, N.R., Hoover, D.G., Knorr, D., 1992. Antibacterial action of chitosan. Food Biotechnology 6, 257–272.

Takahashia, T., Imaia, M., Suzukia, I., Sawai, J., 2008. Growth inhibitory effect on bacteria of chitosan membranes regulated by the deacetylation degree. Biochemical Engineering Journal 40, 485–491.

Takemono, K., Sunamoto, J., Askasi, M., 1989. Polymers and Medical Care. . Mita, Tokyo; 1989; Chapter IV.

Tikhonov, V.E., Stepnova, E.A., Babak, V.G., Yamskov, I.A., Palma-Guerrero, J., Jansson, H.B., Lopez-Llorca, L.V., Salinas, J., Gerasimenko, D.V., Avdienko, I.D., Varlamov, V.P., 2006. Bactericidal and antifungal activities of a low molecular weight chitosan and its N-/2(3)-(dodec-2-enyl)succinoyl/-derivatives. Carbohydrate Polymers 64, 66–72.

Tokura, S., Ueno, K., Miyazaki, S., Nishi, N., 1997. Molecular weight dependent antimicrobial activity by chitosan. Macromolecular Symposia 120, 1–9.

Tsai, G.J., Su, W.H., 1999. Antibacterial activity of shrimp chitosan against *Escherichia coli*. Journal of Food Protection 62, 239–243.

Uchida, Y., Izume, M., Ohtakara, A., 1989. In: Skjak-Braek, G., Anthonsen, T., Sandford, P. (Eds.), Chitin and chitosan. Elsevier, London, UK, p. 373.

Ueno, K., Yamaguchi, T., Sakairi, N., Nishi, N., Tokura, S., 1997. In: Domard, A., Roberts, G.A.F., Varum, K.M. (Eds.), Advances in chitin science. Jacques Andre, Lyon, p. 156.

Wang, X.H., Du, Y.M., Liu, H., 2004. Preparation, characterization and antimicrobial activity of chitosan-Zn complex. Carbohydrate Polymers 56, 21–26.

Weiner, M.L., 1992. In: Brine, C.J., Sandford, P.A., Zikakis, J.P. (Eds.), Advances in chitin and chitosan. Elsevier, London, p. 663.

Wu, Y.B., Yu, S.H., Mi, F.L., Wu, C.W., Shyu, S.S., Peng, C.K., Chao, A.C., 2004. Preparation and characterization on mechanical and antibacterial properties of chitosan/cellulose blends. Carbohydrate Polymers 57, 435–440.

Xie, Y.J., Liu, X.F., Chen, Q., 2007. Synthesis and characterization of water-soluble chitosan derivate and its antibacterial activity. Carbohydrate Polymers 69, 142–147.

Xing, K., Chen, X.G., Liu, C.S., Cha, D.S., Park, H.J., 2009a. Oleoyl-chitosan nanoparticles inhibits *Escherichia coli* and *Staphylococcus aureus* by damaging the cell membrane and putative binding to extracellular or intracellular targets. International Journal of Food Microbiology 132, 127–133.

Xing, K., Chen, X.G., Kong, M., Liu, C.S., Cha, D.S., Park, H.J., 2009b. Effect of oleoyl-chitosan nanoparticles as a novel antibacterial dispersion system on viability, membrane permeability and cell morphology of *Escherichia coli* and *Staphylococcus aureus*. Carbohydrate Polymers 76, 17–22.

Yamada, K., Akiba, Y., Shibuya, T., Kashiwada, A., Matsuda, K., Hirata, M., 2005. Water purification through bioconversion of phenol compounds by tyrosinase and chemical adsorption by chitosan beads. Biotechnology Progress 21, 823–829.

Yancheva, E., Paneva, D., Maximova, V., Mespouille, L., Dubois, P., Manolova, N., Rashkov, I., 2007. Polyelectrolyte complexes between (cross-linked) N-carboxyethylchitosan and (quaternized) poly[2-(dimethylamino)ethyl methacrylate]: preparation, characterization, and antibacterial properties. Biomacromolecules 8, 976–984.

Yang, J.M., Lin, H.T., 2004. Properties of chitosan containing PP-g-AA-g-NIPAAm bigraft nonwoven fabric for wound dressing. Journal of Membrane Science 243, 1–7.

Yang, J.M., Su, W.Y., Leu, T.L., Yang, M.C., 2004. Evaluation of chitosan/PVA blended hydrogel membrane. Journal of Membrane Science 236, 39–51.

Yang, T.C., Chou, C.C., Li, C.F., 2005. Antibacterial activity of N-alkylated disaccharidechitosan derivatives. International Journal of Food Microbiology 97, 237–245.

Yang, T.C., Li, C.F., Chou, C.C., 2007. Cell age, suspending medium and metal ion influence the susceptibility of *Escherichia coli* O157:H7 to water-soluble maltose chitosan derivative. International Journal of Food Microbiology 113, 258–262.

Yang, X.M., Liu, Q., Chen, X.L., Yu, F., Zhu, Z.Y., 2008. Investigation of PVA/ws-chitosan hydrogels prepared by combined γ-irradiation and freeze-thawing. Carbohydrate Polymers 73, 401–408.

Ye, W.J., Leung, M.F., Xin, J., Kwong, T.L., Lee, D.K.L., Li, P., 2005. Novel core-shell particles with poly(n-butyl acrylate) cores and chitosan shells as an antibacteial coating for textiles. Polymer 46, 10538–10543.

Yu, S.H., Mi, F.L., Shyu, S.S., Tsai, C.H., Peng, C.K., Lai, J.Y., 2006. Miscibility, mechanical characteristic and platelet adhesion of 6-O-carboxymethylchitosan/polyurethane semi-IPN membranes. Journal of Membrane Science 276, 68–80.

Zhong, Z.M., Xing, R.G., Liu, S., Wang, L., Cai, S.B., Li, P.C., 2008. Synthesis of acyl thiourea derivatives of chitosan and their antimicrobial activities in vitro. Carbohydrate Research 343, 566–570.

Zivanovic, S., Basurto, C.C., Chi, S., Davidson, P.M., Weiss, J., 2004. Molecular weight of chitosan influences antimicrobial activity in oil-in-water emulsions. Journal of Food Protection 67, 952–959.

Zivanovic, S., Chi, S., Draughon, A.F., 2005. Antimicrobial activity of essential oils incorporated in chitosan films. Journal of Food Science 2005 (70), 45–51.

Zivanovic, S., Li, J.J., Davidson, P.M., Kit, K., 2007. Physical, mechanical and antimicrobial properties of chitosan/PEO blend films. Biomacromolecules 8, 1505–1510.