ADVANCES IN CHITIN SCIENCE

Volume XII

Proceedings of the 5th Iberoamerican Chitin Symposium

06 – 09 June 2010
Santiago do Chile
– Extended Abstracts –

Organized by

Dr. Telma T. Franco
Professor of Chemical Engineering
V SIAQ Chair and President of SIAQ
State University of Campinas, Brazil

Dr. Martin G. Peter
Emeritus Professor of Natural Products
University of Potsdam, Germany
and
Visiting Professor
State University of Campinas, Brazil

Antimicrobial analysis of gels and films from chitosan and *N,N,N*-trimethyl chitosan

Rejane C. Goy, Douglas de Britto, Odilio B. G. Assis*

Embrapa Instrumentação Agropecuária, Rua XV de Novembro, 1452, 13560-970 São Carlos, SP, Brazil
*E-mail: odilio@cnpdia.embrapa.br

Abstract
Chitosan and its derivatives have been explored as bactericidal and fungicidal agent in the inhibition against a large number of fungi and bacteria both gram-negative and gram-positive. Some antimicrobial mechanisms have been proposed being the most acceptable the presence of NH^{+3} and its interactions with the bacteria surface constituents. In this study the effectiveness of chitosan and water-soluble N,N,N-trimethyl chitosan against *Staphylococcus aureus* (gram-positive bacteria) and *Escherichia coli* (gram-negative bacteria) were evaluated in gel and cast film forms. The results show better activity against gram-negative microorganism, with influence of polymer concentration mainly for quaternized chitosan derivative.
Keywords: Antibacterial activity, Chitosan, Water-soluble chitosan; S. *aureus*; E. *coli.*

INTRODUCTION

Due the easily capacity to form gels and be transformed in films with good mechanical properties, chitosan has a potential for applications in several technological fields, such as in agriculture, food, medicine, biotechnology, textiles, polymers, and watertreatment. One important application area is its use as protective edible coating of natural products and foods. Chitosan coatings act against the development of microorganisms and also assists in the control of physiological, morphological and physicochemical changes.[1,2] Chitosan can be considered either bactericidal (killing the microorganisms) or bacteriostatic (preventing or inhibiting their growth), but hardly distinguished between the two mechanisms.

The N,N,N-trimethyl chitosan (TMC) is a partially quaternized derivative of chitosan synthesized to increase solubility in water at neutral or basic pH values, and thereby increasing the applications capability. TMC has several advantages upon the parent polymer where *in vivo* studies have shown that properties like absorption and biocompatibility are quite enhanced.[3]

In the TMC, the addition of permanent positive charges in the chains can be attained through the synthesis of quaternary chitosan salts - via the covalent addition of a substituent containing a quaternary ammonium group,[4] or by the quaternization of the amino groups in the parent polymer.[5] These quaternary salts have also shown to have greater bactericidal efficiency than the primary chitosan, since an increasing in amount of cationic sites will promote a better electrostatic interaction with microorganisms walls.[6,7] In function of their outer membrane composition, it has been demonstrated that hydrophilicity in gram-negative bacteria is significantly higher than in gram-positive bacteria, making them most sensitive to chitosan.[8] Additionally, the charge density on the bacteria cell surface also to play an important role in determining the amount of adsorbed chitosan. More adsorbed chitosan on hydrophilic surface would result in greater changes in the wall structure and consequently altering cell membrane permeability.[9] Such feature can explain, in part, why some gram-negative bacteria are most sensitive to chitosan.

In this study the effectiveness of chitosan and water-soluble N,N,N-trimethyl chitosan against gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*) were

assessed in function of the polymer concentration in gel and in film forms.

MATERIALS AND METHODS

The starting chitosan was medium molar weight purchased from Sigma-Aldrich (Brazil). For methylation reaction, the basic sequence consisted in an initial suspension of 1 g of chitosan (0.005 mol) in 16 cm^3 of dimethylsulfate (Synth, Brazil) and 4 cm^3 of deionized water. 1.2 g of NaOH (0.015 mol) and 0.88 g of NaCl (0.015 mol) were added and the solution mixed. The final product was obtained by precipitation with acetone. After rinsing, the derivative was filtered and vacuum dried. Methylation details and TMC characterization can be found elsewhere[5]. Gels were prepared by dissolving the commercial chitosan in 1% acetic acid in deionized water with constant stirring for 2 hours. Metlylated salt was directly dissolved in water. Concentrations of 0.5, 1.0, 2.0, 3.0 and 4.0 gL^{-1} were prepared for both materials. Films were then prepared by solution casting onto an acrylic plate. Solvents were allowed to evaporate at room temperature. After drying the films were peeled from the plate.

The antibacterial activity was evaluated according to the inhibitory halo area method. For that Petri dishes containing TSB (Tryptic Soy Broth) and agar medium were prepared and *E. coli* and S. *aureus* microorganisms inoculated after appropriated dilution. For gels testing parts of the medium were removed to form small holes where the gels were placed after the bacteria were inoculated. For films testing, pieces of them were cut and placed on the surface of culture medium previously inoculated with the microorganisms. The Petri dishes were left overnight inside a circulation oven for the bacteria to grow at 32-37 °C and inhibition zones measured on bases in the average diameter of the clear inhibition zone directly on the dishes.

RESULTS AND DISCUSSION

Figure 1 illustrates the antimicrobial activities by formation of inhibition zones for films of chitosan and TMC, both processed at a concentration of 2.0 g × L^{-1}. Both materials present an effective antibacterial activity with larger zone to TMC in comparison to chitosan. For both type of bacteria tested inhibition zones around the films are visualized mainly for *S. aureus* growth whereas for *E. coli* no clear microbial inhibitions are evidenced. Similar results are obtained in gel form materials.

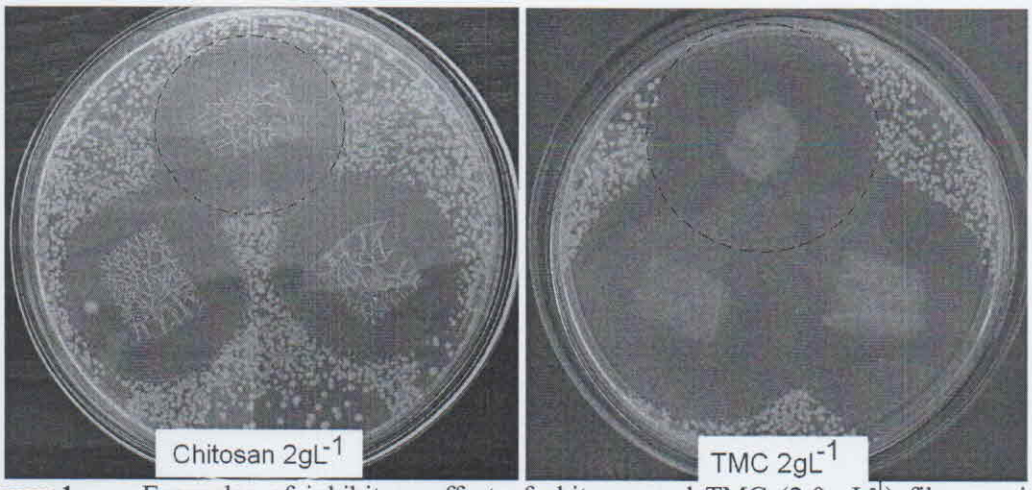

Figure 1. Examples of inhibitory effect of chitosan and TMC (2.0 gL^{-1}) films against gram-positive bacteria *S. aureus*.

These results in some way confirm a better antibacterial activity against gram-positive bacteria (*S. aureus*) is more effective than gram-negative (*E. coli*), which should be attributed to their different cell walls. In the gram-positive bacterium, the cell wall is fully composed of

peptide polyglycogen. According to Xie *et al.*[10] the peptidoglycan layer is composed of networks with plenty of pores, which allow foreign molecules to come into the cell without difficulty. But for *E. coli*, the cell wall is made up of a thin membrane of peptide polyglycogen and an outer membrane constituted of lipopolysaccharide, lipoprotein, and phospholipid. In such bilayer structure the outer membrane has high molecular weight and acts as a barrier against foreign molecules.

The film concentration also appears to play an important role in the antimicrobial activity, mainly for the TMC. For this derivative the measured inhibition zone increases exponentially with the concentration stabilizing up to 2 gL^{-1} (Figure 2). Further increases in the concentration of TMC result in no substantial improvement in the activity. Conversely, for commercial chitosan the antibacterial activity is confirmed to be inferior and reduces slightly as the concentration increases.

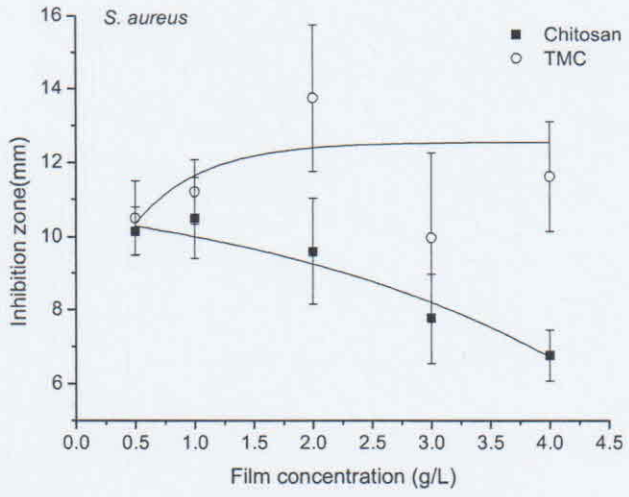

Figure 2. Inhibition zone in function of the film concentration, as measured against the bacteria *S. aureus.*

The inferior inhibitory effect observed to chitosan can be attributed to several factors though it is supposed that the relatively small number of charged amino groups in the molecules can be a determinant feature. In the quaternized salt positive charges are permanent in the polymer chains what results in better electrostatic interaction with the microorganisms walls and consequently a relatively higher antimicrobial activity. From the present tests it is not possible to determine an exact antimicrobial mechanism, anyway the effectiveness of water soluble chitosan (TMC) is evident.

CONCLUSIONS

The analyzed materials show effective antimicrobial activity against mainly gram-positive bacteria (*S. aureus*). The chitosan quaternary salt shown superior antimicrobial activity when compared with the chitosan and the material concentration in the films manufacture is important parameter, since increasing the film concentration also increases the inhibition zone formed but the same do no occurs with the chitosan, where the concentration is not so important, besides the inhibition is so effective too.

ACKNOWLEDGEMENTS

This work was supported by FAPESP and Embrapa (Rede AgroNano).

REFERENCES

1 Sharidi F, Arachchi JKV and Jeon Y-J. *Trends Food Sci Techn* **10**: 37-51(1999)

2 Britto D and Assis OBG. *Biol Macrom* **41**: 98-203 (2007).

3 Chen F, Zhang ZR, Yuan F, Qin X, Wang M and Huang Y. *Int J Pharm* **349**:226-33 (2008).

4 Curti E, Britto D and Campana-Filho SP. *Macromol Biosci* **3:**571-576(2003).

5 Britto D and Assis OBG. *Carbohyd Polym* **69:** 305-310(2007).

6 Simpson BK, Gagne N, Ashie INA and Noroozi E. *Food Biotechn* **11:** 25-44(1997).

7 Chung Y-C, Su Y-P, Chen C-C, Jia G, Wang H-L, Wu JCG and Lin J-G. *Acta Pharmacol Sinica* **25:**932-936 (2004).

8 Másson M, Holappa J, Hjálmarsdóttir M, Rúnarsson ÖV, Nevalainen T and Järvinen T. *Carbohyd Polym* **74:** 566–571(2008).

9 Goy RC, Britto D and Assis OBG. *Polímeros: Ciênc Tecnol*. 19:231-237(2009).

10 Xie W, Wang W and Liu Q. *Carbohyd Polym* **50:** 35-40(2002).