HACETTEPE JOURNAL OF BIOLOGY AND CHEMISTRY Research Article
Hacettepe J. Biol. & Chem., 2009, 37 (4) 353-357

In-Vitro Characterization of Antimicrobial Effect of Polyvinylimidazole

Ozan Gurbuz[1]*, Yasemin Sahan[1], Ali Kara[2], Bilgen Osman[2]

[1]Uludag University, Department of Food Engineering, Bursa, Turkey

[2]Uludag University, Department of Chemistry, Bursa, Turkey

Article Info

Article history:

Received
October 12, 2009

Received in revised form
November 27, 2009

Accepted
December 13, 2009

Available online
December 31, 2009

Key Words

Polyvinylimidazole, Antimicrobial effect, *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Candida albicans, Kloecera apiculata, Aspergillus niger*, *Penicillium roqueforti*

Abstract

The present study was devised to describe the effectiveness of degradable polymer (polyvinylimidazole, PVI) on microbial growth in-vitro. We observed the antimicrobial outcome of PVI on fungi and pathogen bacteria that are found in food-borne disease. The following pathogens and fungi examined were: *Escherichia coli* (ATCC 35218), *Salmonella enteritidis* (ATCC 13076), *Staphylococcus aureus* (ATCC 33862) as bacterial organisms; *Candida albicans, Kloeckera apiculata* as yeast organisms; *Aspergillus niger* and *Penicillium roqueforti* as fungi. We utilized a plate count method with the test microorganisms. PVI, as a non-modified polymer, exhibited an inhibition effect on all test microorganisms. Currently there is only theoretical conjecture on the specific role PVI plays as an inhibition to food-borne fungi and pathogens. A comparison of the sensitivity of these microorganisms to PVI showed the greatest inhibitive effect was found with *Kloeckera apiculata* followed by *Candida albicans*. Both types of yeast completely died in 1 mg/mL concentration of PVI during 12-24 h treatments. Weaker activity is shown against *Aspergillus niger* and *Penicillium roqueforti.* These results were comparable with three different concentrations of PVI. The polymer was less effective, in a descending order, against *Salmonella enteritidis*, *Escherichia coli* and *Staphylococcus aureus*, respectively. Similar effects after 24 h incubation were observed (R^2=1).

INTRODUCTION

Antimicrobial polymers have been widely used for the preservation of food quality and freshness as well as for the control of microbiological growth. Recently some polymeric compounds, GRAS (Generally Regarded As Safe), non-GRAS and natural antimicrobial compounds, have become important for inclusion within food packaging material. The antioxidant and antimicrobial properties of these various polymers have been of great interest because of their possible use as food-coating materials for the prevention of oxidation, controlling pathogens and/or toxin-producing microorganisms in foods [1-4]. This interest has emerged from a growing propensity to replace synthetic polymers with biocompatible or biodegradable ones.

Although the antimicrobial activity of polymers was

* Correspondence to: Ozan Gürbüz,

Uludag University, Department of Food Engineering, Bursa, Turkey

Tel: +90224 294 1500 Fax: +90224 294 1402
E-mail: ozang@uludag.edu.tr

recognized long ago, their application as coating additives has received increased attention in the food industry. Recent studies have shown that microorganisms such as strains of *Escherichia coli* are among the most active pathogens [5-7]. The aim of this study was to characterize the antimicrobial activity of the polymer obtained from PVI against *Escherichia coli* (ATCC 35218)*, Salmonella enteritidis* (ATCC 13076)*, Staphylococcus aureus* (ATCC 33862) as bacteria*; Candida albicans, Kloeckera apiculata* as yeast organisms; and *Aspergillius niger* and *Penicillium roqueforti* as fungi.

MATERIAL AND METHODS

Microorganism Strains

Escherichia coli (ATCC 35218), *Salmonella enteritidis* (ATCC 13076), *Staphylococcus aureus* (ATCC 33862) strains were purchased from Oxoid (Wesel, Germany); *Candida albicans, Kloeckera apiculata, Aspergillus niger* and *Penicillium roqueforti* were obtained from Department of Food Engineering, Faculty of Agriculture of Uludag University, Bursa, Turkey.

Polyvinylimidazole (PVI)

The polymer was synthesized by a free-radical polymerization in benzene using azoisobutyronitrile as an initiator, as previously reported [8-10].

Antimicrobial assay

Antimicrobial activity of the polymer was tested by the plate count method [11]. The stock cultures of bacteria were maintained on Nutrient Agar (Oxoid, Ltd, Hampshire, England), yeasts and molds on Sabouraud Dextrose Agar (Difco, Detroit, USA) at 4ºC until used. Each of the bacteria, yeasts and molds were sub-cultured. Colonies were then transferred aseptically from the second transfer plate into individual tubes containing sterile nutrient and Sabouraud broth, respectively. The tubes were incubated for a period of 24 h at 37ºC for bacteria and 30ºC for yeasts and fungi to ensure that the microorganisms were in the log phase. The polymer studied was in concentrations of 0.5, 1 and 2 mg/mL. Subsequently, the microorganism suspensions were visually adjusted to 0.5 McFarland and then further diluted 1:100 with fresh sterile broth to yield starting inoculums of approximately 10^7 CFU/mL (bacteria) and 10^6 CFU/mL (yeasts and molds). All plates were incubated at suitable temperatures for 1, 12 and 24 h and colonies were counted. Tests were carried out in triplicate.

RESULT AND DISCUSSION

In general PVI, as a non-modified polymer, exhibited an inhibition effect on all microorganisms tested (Table 1). Currently, there is only theoretical conjecture on the specific role PVI plays as an inhibition of food-born fungi and pathogens.

The antimicrobial activity of the polymers may be accounted for by the contribution of the polymers to each elementary process in the lethal action. This might be thought that the polymer inhibited microorganisms by adsorption onto the bacterial cell surface, diffusion through the cell wall, adsorption onto the cytoplasmic membrane, disruption of the cytoplasmic membrane, leakage of the cytoplasmic constituents, and death of the cell [12].

A comparison of the sensitivity of these microorganisms to PVI showed the greatest inhibition effect was on *Kloeckera apiculata* followed by *Candida albicans.* Both types of yeast died completely in 1 mg/mL concentration of PVI during 12-24 h treatments. Similar observations were found by Meng et al., (2009) for *Candida albicans*. These researchers reported that the antifungal activity of the the poly(dimethylsiloxane) (PDMS)/mont-

Table 1. The inhibition level in different concentration of the PVI tested against pathogen microorganisms

Microorganisms	Source	Time(h)	Concentration (mg/mL)		
			0.5	1	2
E. coli	ATCC 35218	1	6.48	6.03	5.46
		12	6.12	5.46	3.80
		24	6.07	4.07	3.54
S. aureus	ATCC 33862	1	7.00	6.69	5.69
		12	7.00	5.75	5.40
		24	7.00	5.43	4.69
S. enteritidis	ATCC 13076	1	5.79	5.61	5.23
		12	5.20	3.61	4.06
		24	5.11	3.11	3.78
C. albicans	ATCC 102 31	1	5.47	-	-
		12	3.74	-	-
		24	3.00	-	-
*K. apiculata**	UUFE-KA11	1	3.00	-	-
		12	1.92	-	-
		24	1.62	-	-
*A. niger**	UUFE-AN07	1	6.00	5.92	5.67
		12	6.00	5.70	5.45
		24	6.00	5.65	5.13
*P. roqueforti**	UUFE-PR21	1	6.00	5.84	5.70
		12	6.00	5.60	5.51
		24	6.00	5.37	5.05

*:This microorganisms were obtained from the Food Engineering Department, Uludag University (UUFE).

morillonite–terbinafine hydrochloride (PDMS/OMMT) nanocomposite films was evaluated by the inhibitory zone tests and revealed strong activity against *Candida albicans* [13].

All three-polymer concentrations exhibited antimicrobial activity against *Escherichia coli, Salmonella enteritidis* and *Staphylococcus aureus* (Figures 1-3). Weaker activity was shown against *Aspergillius niger* and *Penicillium roqueforti* (Table 1).

Antimicrobial activity was slightly stronger with higher concentrations of the polymer. However, similar results were found with all three PVI concentrations.

In descending order, the polymer became less effective against *Salmonella enteritidis*, *Escherichia coli* and *Staphylococcus aureus*, respectively. Similar effects were observed after 24 h incubation (R^2=1). In others works, researchers reported that various inhibition level for these bacteria used different polymers.

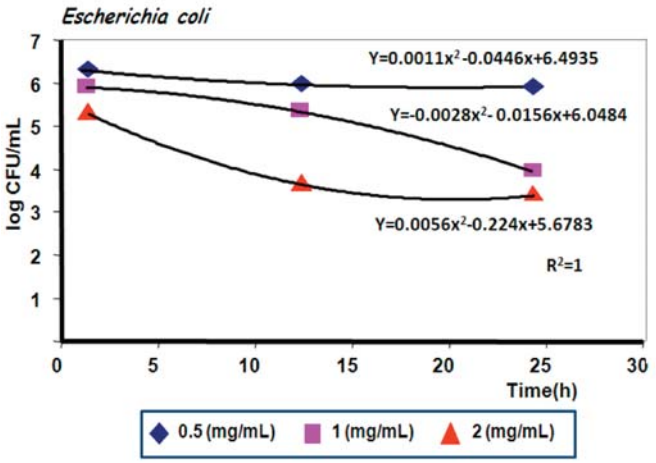


Figure 1. Antimicrobial activity of PVI against *E. coli* O157:H7.

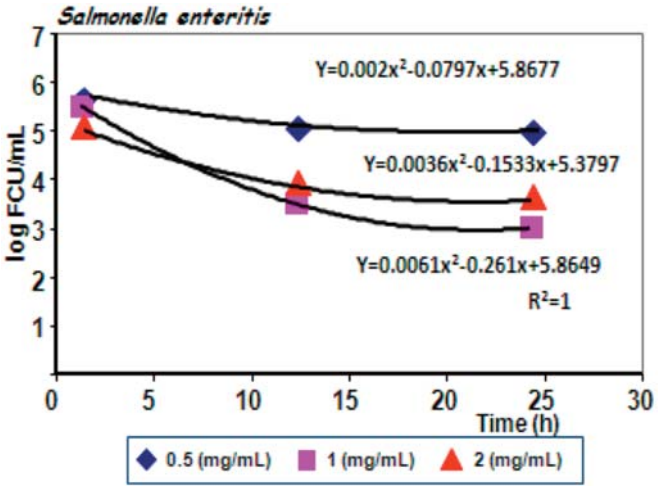


Figure 2. Antimicrobial activity of PVI against *Salmonella enteritidis.*

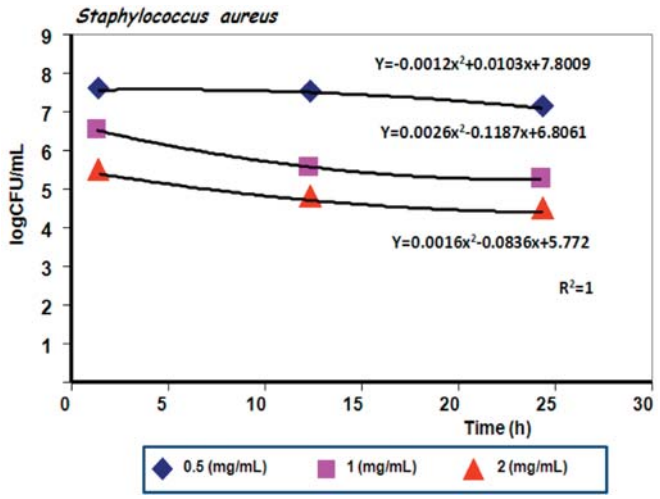


Figure 3. Antimicrobial activity of PVI against *S. aureus.*

With *Staphylococcus aureus*, Tiller et al. (2001) found that pyridine groups N-alkylated with C6 showed the highest killing efficacy, followed by those with C3 and C4 chains, and the C8-C16 chains are significantly less effective [14].

Hu et al., (2005) showed that the pyridinium groups bearing alkyl substituents of 4-10 carbons in length are highly effective in disrupting the cell membrane of the *Escherichia coli*. The beads N-alkylated with C4 and C6 chains have a high surface concentration of pyridinium groups. As such, an amount of beads of 0.8% (w/w) of an *Escherichia coli* suspension of 10^5 CFU/mL can result in almost 100% killing efficiency within 20 min [15].

Kapadia et al., (2009) showed that the Ln(III) coordination polymers acted as an efficient and effective catalysts and antimicrobial agents. The insertion of metal ion in the polymeric ligand enhances antimicrobial activity significantly against *Escherichia coli* and *Staphylococcus aureus* [16].

Kenawy et al., 2006, evaluated of the antimicrobial activity of cross linked copolymers based on copolymerization of vinylbenzyl chloride (VBC) either with 2-chloroethyl vinyl ether (CEVE), or methylmethacrylate (MMA) using the divinylbenzene as cross linker (DVB). The antimicrobial activity of the modified copolymers was tested against *Candida albicans* SC5314, *Escherichia coli* and *Staphylococcus aureus*. The copolymers showed antimicrobial activity against the tested microorganisms, but the compound with the triphenylphosphonium salt of the modified copolymer being the most effective on bacteria and fungi species [3].

The fungi used in this study were the most resistant to PVI. There was no inhibition effect with 0.5 mg/L concentration in all three time periods (1,12,24 h). At higher concentrations of PVI, *Aspergillius niger* continued to be the most resistant. Similar results were found by Ziani et al., (2009) [17].

Ziani et al., (2009) also reported differences on the effectiveness of chitosan as antifungal agent against *Aspergillius niger* [17]. However, Sebti et al. (2005) and Plascencia- Jatomea et al., (2003) showed that the inhibition growth of *Aspergillius niger* was improved using chitosan with different molecular weight and different condition [18,19]. In addition Agullo et al. (2003) observed that the use of a chitosan-based coating delayed *Penicillium* spp., growth [20].

A comparison of the antibacterial and antifungal assays shows that the inhibitory effect of the polymer on the growth of fungi is not as effective as bacteria. It is known that fungal spores have very tough spore coats and more components (glucans, mannans, chitin, etc.) are present in the cell wall of fungi compared with that of bacteria (mainly peptidoglycan) [15] which may increase the difficulty for the PVI to penetrate into the cell body.

CONCLUSION

Results show that PVI exhibited significant antimicrobial properties. It should be mentioned that the killing potency depends on both the chemistry of the polymer as well as the structure of the microorganism. These properties may prove to be beneficial in the future for the preservation and/or extension of the shelf life of raw as well as processed foods. A further application could be in the area of pharmaceuticals for the protection of human life. Additional work should be done to explore other new antimicrobial polymer substances present in the chemical industry.

REFERENCES

1. Quintavalla, S., Vicini, L., Antimicrobial food packaging in meat industry. Meat Sci., 62, 373-380, 2002.
2. Rabea, E.I., Badawy, M.E.T., Stevens, C.V., Smagghe, G., Steurbaut, W. Chitosan as antimicrobial agent: applications and mode of action, Biomacromol., 4(6), 1457-1465, 2003.
3. Kenawy, E.R., Worley, D., Broughton, R., The chemistry and applications of antimicrobial polymers: a state-of-the-art review. Biomacromol., 8(5), 1359-1383, 2007.
4. Zivanovic, S., Chi, S., Draughon, A.F. Antimicrobial activity of chitosan films enriched with essential oils. J Food Sci., 70(1), M45-M51, 2005.
5. Elgayyar, M., Draughon, F.A., Golden, D.A., Mount, J.R., Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J Food Prot., 64, 1019-1024, 2001.
6. Dorman, H.J.D., Deans, S.G., Antimicrobial agents from plants: antibacterial activity of plant volatile oils, J. Appl. Microbiol., 88, 308–316, 2000.
7. Burt, S.A., Reinders, R.D., Antibacterial activity of selected plant essential oils against Espherichia coli 0157:H7, Lett. Appl. Microbiol., 36(3), 162-167, 2003.
8. Böhmer, M., Heesterbeek, W.H.A., Deratani, A., Renard, E. Adsorption of partially quarternied poly(vinylimidazoles) onto SiO_2 and Y_2O_3, Colloid. Surf. A: Physicochem. Eng. Aspects, 99, 53-64, 1995.
9. Pekel, N., O. Güven, Investigation of complex formation between poly(N-vinyl imidazole) and various metal ions using the molar ratio method, Colloid Polym. Sci. 277, 570-573, 1999.
10. Tekin, N., Kadıncı, E., Demirbaş, Ö., Alkan, M., Kara, A., Doğan, M., Surface properties of poly(vinylimidazole)-adsorbed expanded perlite, Micropor. Mesopor. Mater. 93,125-133, 2006.
11. Anonymous. Aerobic colony count by pour plate method. HPA Standard Method. Standards Unit, Evaluations and Standards Laboratory in Conjunction with the Reginal Food, Water and Environmental Coordinators Forum. Issue 4.2, p14, 2008.
12. Kenawy, E.R., Abdel-Hay, F.I., El-Magd, A.A., Mahmoud, Y. Biologically active polymers: VII. Synthesis and antimicrobial activity of some crosslinked copolymers with quaternary ammonium and phosphonium groups. React. Functl. Polym., 66, 419-429, 2006.
13. Meng, N., Zhou, N.L., Zhang, S.Q., Shen, J. Synthesis and antifungal activities of polymer/montmorillonite-terbinafine hydrochloride nanocomposite films. Appl. Clay Sci., 46, 136-140, 2009.
14. Tiller J.C., Liao C.J., Lewis K., Klibanov, A.M. Designing surfaces that kill bacteria on contact. PNAS, USA, 98, 5981-5985, 2001.
15. Hu, F.X., Neoh, K.G., Cen, L., Kang E.T. Antibacterial and antifungal efficacy of surface functionalized polymeric beads in repeated applications. Biotechnol. Bioeng., 89(4), 474-484, 2005.
16. Kapadia, M.A., Patel, M.M., Joshi, J.D. Coordination polymers of Ln(III): Synthesis, characterization, catalytic and antimicrobial aspects. Inorg. Chim. Acta, 362, 3292-3298, 2009.
17. Ziani, K., Fernandez-Pan, I., Royo, M., Mate, J.I. Antifungal activity of films and solutions based on chitosan against typical seed fungi. Food Hydrocolloids 23, 2309-2314, 2009.
18. Sebti, I., Martial-Gros, A., Carnet-Pantiez, A., Grelier, S., Coma, V. Chitosan polymer as bioactive coating and film against *Aspergillus niger* contamination. J. Food Sci., 70(2), 100-104, 2005.
19. Plascencia-Jatomea, M., Viniegra, G., Olayo, R., Castillo-Ortega, M. M., Shirai, K. Effect of chitosan and temperature on spore germination of *Aspergillus niger*. Macromol. Biosci., 3, 582-586, 2003.
20. Agullo, E., Rodriguez, M.S., Ramos,V., Albertengo, L. Present and future role of chitin and chitosan in food. Macromol. Biosci., 10, 521-530, 2003.