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Production of Antimicrobial Films by Incorporation of Partially Purified Lysozyme into Biodegradable Films of Crude Exopolysaccharides Obtained from *Aureobasidium pullulans* Fermentation

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Summary

Antimicrobial films were produced by incorporating partially purified lysozyme into films of crude exopolysaccharides (59 % pullulan) obtained from *Aureobasidium pullulans* fermentation. After film making, the films containing lysozyme at 100, 260, 520 and 780 μg/cm^2 showed 23 to 70 % of their expected enzyme activities. The highest recovery of enzyme activity (65–70 %) after the film making was obtained in films prepared by incorporating lysozyme at 260 μg/cm^2 (1409 U/cm^2). The incorporation of disodium EDTA·2H_2O and sucrose did not affect the initial lysozyme activity of the films significantly. With or without the presence of disodium EDTA·2H_2O at 52 or 520 μg/cm^2, lysozyme activity showed sufficient stability in the films during 21 days of cold storage. However, the presence of sucrose at 10 mg/cm^2 in the films caused the destabilization of part of enzyme activity (almost 35 %) at the end of storage. The combinational incorporation of lysozyme at 780 μg/cm^2 (4227 U/cm^2) and disodium EDTA·2H_2O at 520 μg/cm^2 gave antimicrobial films effective on *Escherichia coli*. However, in the studied lysozyme concentration range the films did not show any antimicrobial activity against *Lactobacillus plantarum*. This study clearly showed that the partially purified lysozyme and crude exopolysaccharides from *Aureobasidium pullulans* may be used to obtain antimicrobial films to increase the safety of foods.

Key words: lysozyme, exopolysaccharides, pullulan, *Aureobasidium pullulans*, antimicrobial films

Introduction

The increased demand for safe and minimally processed fresh produce has intensified the research on antimicrobial packaging. Different chemicals such as organic or inorganic acids, metals, alcohols, ammonium compounds or amines can be incorporated into packaging materials as antimicrobials (*1,2*). However, because of the health concerns of the consumers, packaging industry shows greater interest in the use of biopreserva-

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tives for antimicrobial packaging. The main biopreservatives suggested for this purpose are bacteriocins such as nisin, pediocin and lacticin and antimicrobial enzymes such as lysozyme, lactoperoxidase, chitinase and glucose oxidase (*2,3*).

One of the potential biopreservatives to be used in antimicrobial packaging is lysozyme (*1,2,4*). This enzyme shows antimicrobial activity mainly on G(+) bacteria by splitting the bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine of the peptidoglycan in their cell walls. Furthermore, when it is combined with EDTA, which destabilizes the outer membrane of G(–) bacteria, the antimicrobial spectrum of the lysozyme increases significantly (*5,6*). Because of the environmental concerns and technological problems associated with the hydrophobic nature of most plastic films and the denaturing effect of thermal polymer processing methods, such as extrusion and injection molding, the incorporation of lysozyme and other biopreservatives into films made from biodegradable materials attracts a great interest (*1,2,4*). Most of these materials are edible and their film formation occurs under mild conditions. Different edible films tested for this purpose include zein, methylcellulose, hydroxypropyl methylcellulose, carrageenan, alginate, and whey proteins (*2,4,5,7–10*).

An alternative source for biodegradable films are fungal polysaccharides. For example, extensive studies related to the exopolysaccharides obtained from the polymorphic fungus *Aureobasidium pullulans* have long been conducted (*11*). The main polysaccharide formed by *A. pullulans*, generally regarded to be non-pathogenic and non-toxigenic, is pullulan (*12*). However, depending on the fermentation conditions and the ratio of the morphological forms of *A. pullulans*, different β-glucans, acidic polysaccharides and heteropolysaccharides that have not been fully characterized may also be formed in the fermentation medium (*13*). Pullulan is an unbranched homopolysaccharide that is formed by maltotriose and maltotetraose units having both α(1→6) and α(1→4) linkages. It is one of the few neutral, water-soluble microbial polysaccharides that form excellent soluble and transparent films with relatively low oxygen permeability and it can be produced in large quantities by fermentation (*14*). However, because of different problems associated with pullulan production this polysaccharide has not found a word-wide interest by food industry and bioindustry. The major problems associated with pullulan production are: (*i*) costs associated with the purification of pullulan from the culture broth that may contain different polysaccharides and brown coloured melanins produced by *A. pullulans* (*14,15*), (*ii*) changing yields and molecular properties of pullulan affected by the variation of the morphological forms and/or *in situ* exopolysaccharide degrading enzyme systems of *A. pullulans*, and fermentation conditions (*16,17*). The main objective of this study was to use partially purified lysozyme as an antimicrobial in films of crude exopolysaccharides obtained from a melanin deficient *A. pullulans* strain. The study was focused mainly on determining the recovery of the activity of the enzyme in films, cold storage stability of the enzyme activity in films containing different ingredients and antimicrobial activity of films on selected microorganisms. Partially purified lysozyme and crude exopolysaccharides used in this study were obtained with fast, simple and inexpensive fractionation methods. Thus, the antimicrobial films developed can be more easily adapted to food industry for the coating of suitable food products.

Materials and Methods

Materials

Micrococcus lysodeikticus cells and dialysis tubes (12000 MW, prepared as described in the manual) were obtained from Sigma Chem. Co. (St. Louis, Mo., USA). Disodium EDTA·$2H_2O$ was purchased from Riedel-de Haën (Sigma-Aldrich Laborchemikalien, Seelze, Germany). Fresh hen eggs used to produce lysozyme were obtained from a supermarket in Izmir (Turkey). Nutrient agar and MRS agar were obtained from Merck (Darmstadt, Germany) and Oxoid (Hampshire, England), respectively. The nutrient broth (peptone 15 g/L, yeast extract 3 g/L, NaCl 6 g/L, glucose 1 g/L, pH=7.5) and MRS broth (peptone 10 g/L, Lab-Lemco powder 10 g/L, yeast extract 5 g/L, glucose 20 g/L, Tween 80 1 mL/L, K_2HPO_4 2 g/L, sodium acetate 5.0 g/L, triammonium citrate 2 g/L, $MgSO_4·7H_2O$ 0.2 g/L, $MnSO_4·4H_2O$ 0.05 g/L, pH=6.2–6.6) were prepared in the laboratory. The melanin deficient *Aureobasidium pullulans* P56 strain was kindly donated by Prof. Dr. T. Roukas from Aristotle University of Thessaloniki, Greece.

Microorganism and culture conditions

A. pullulans was maintained on PDA slants at 4 °C by subculturing every 3 weeks and prepared for inoculation as described in Göksungur *et al.* (*18*). The inoculation of the production medium was at the level of 5 %.

Fermentation conditions

The production was conducted in a bioreactor (Chemap AG 8708, 7 L capacity, Mannedorf ZH Schweiz, Switzerland). The synthetic fermentation medium was prepared as described by Youssef *et al.* (*15*). Minor modifications of the conditions of fermentation were applied according to Göksungur *et al.* (*18*).

Isolation of exopolysaccharides from fermentation medium

At the 6th day of fermentation, the medium was collected and then clarified by centrifugation at 4000 x *g* for 10 min. To precipitate the formed exopolysaccharides, the supernatant was treated with 2 volumes of ethanol (96 %) and incubated for 1 h at 4 °C. The precipitate was collected by centrifugation and dried in a vacuum oven at 40 °C for 2 days. The fermentation was repeated three times and the obtained dry powders were combined and processed to fine powder by using a home type coffee grinder. The pullulan concentration of this powder was determined as described in Leathers *et al.* (*19*) by assaying the amount of reducing sugars formed by pullulanase (Promozyme D2, Novozymes A/S, Denmark) with DNS method. Pure pullulan used as standard was kindly donated by Hayashibara Biochemical Co. (Japan).

Film making

The obtained fine white powder, containing 59 % of pullulan, was dissolved in distilled water to obtain the final 2.6 % mass per volume ratio of pullulan. After dissolving different ingredients (partially purified lysozyme, disodium EDTA·$2H_2O$ or sucrose), the pH values of the film making solutions were adjusted to 6.5 by 0.1 M NaOH and 1-mL portions of these solutions were pipetted and spread onto plastic petri plates (35 mm in diameter). The films were obtained by drying petri plate contents in an incubator at 25 °C for 21 h. The average thickness of the films determined by a micrometer was 0.032 mm (average of 60 measurements from three films).

Production of partially purified lysozyme

The lysozyme was produced from hen egg white by modifying the partial purification step described by Jiang *et al.* (*20*). This method includes the precipitation of nonlysozyme proteins by incubation with the addition of ethanol following the dilution of the egg white with 0.05 M NaCl solution and adjustment of its pH to 4. Minor modifications made in this method were the change of 8-hour incubation period in the presence of 30 % of ethanol to 6 h and the speed and time of centrifugation applied after incubation to 15 000 x *g* for 15 min at 4 °C. The major modification, on the other hand, was the application of 24-hour dialysis at 4 °C (by three changes of 2000 mL of distilled water) following centrifugation. The activities of lyophilized enzymes prepared by this method were 4767 and 5419 U/mg.

Protein content

Protein content was determined according to Lowry procedure by using bovine serum albumin as standard (*21*).

Lysozyme activity

The activity of lysozyme was determined spectrophotometrically at A_{660nm} by using a Varian (Model Carry 50, Australia) spectrophotometer. The reaction mixture was formed by mixing 0.1 mL of enzyme extract or enzyme containing solution prepared by dissolving films in distilled water and 2.9 mL of *Micrococcus lysodeikticus* cell suspension (0.26 mg/mL, prepared in 0.05 M Na-phosphate buffer at pH=7) at 30 °C. The mixture was rapidly mixed with a vortex and immersed into a water bath at 30 °C. The absorbance of the reaction mixture was determined at the end of 1st minute and the difference between this absorbance value and the initial absorbance value was used for the calculation of enzyme activity (the absorbance-time curves were linear for 1.5–2 min). The enzyme activity was expressed as U (0.001 absorbance change in one minute). The averages of three measurements were used in all tests.

The effect of lysozyme concentration, film ingredients and cold storage on lysozyme activity remained in films

The lyophilized lysozyme was dissolved in film making solutions at different concentrations (1–7.5 mg/mL) to obtain films containing lysozyme at almost 100, 260, 520 and 780 μg/cm^2. The expected lysozyme activity of these films was almost 542, 1409, 2818 and 4227 U/cm^2, respectively. The effects of disodium EDTA·$2H_2O$, used to increase antimicrobial action of lysozyme against G(–) bacteria, were tested by dissolving it in film making solutions at 0.5 and 5 mg/mL to obtain final concentrations of 52 or 520 μg/cm^2 in dried films, respectively. The effect of sucrose, used as a film plasticizer and protein stabilizer, was tested by adding this ingredient to film making solutions at 0.1 g/mL to obtain final concentration of 10 mg/cm^2 in dried films. The remaining lysozyme activity of the obtained films containing different ingredients was tested immediately after drying or periodically during cold storage conducted by keeping nylon wrapped petri plates at 4 °C for 21 days. To determine the remaining lysozyme activity in various films, the films were dissolved in 2 mL of distilled water and 0.1- mL aliquots of this solution were used in the determination of activity as described above. The recovery of lysozyme activity after the film making was determined by calculating the ratio of the remaining lysozyme activity determined for films (U/cm^2) to the expected lysozyme activity incorporated into films (U/cm^2) and multiplying the obtained value by one hundred.

Antimicrobial activity of films

Test of antimicrobial activity was conducted by using *Escherichia coli* (ATCC 53868) or *Lactobacillus plantarum* (DSM 1954) as test microorganisms. The overnight cultures of *E. coli* and *L. plantarum* were prepared in nutrient broth and MRS broth, respectively. All incubations were conducted at 37 °C. For antimicrobial tests, 9 discs (0.9 cm in diameter) prepared from each film by a cork borer under aseptic conditions were applied to microorganisms. For *E. coli* (cell concentration of culture was almost $1.8 \cdot 10^8$ CFU/mL), three discs were placed carefully onto each nutrient agar plate with 0.1 mL of culture. For *L. plantarum* (cell concentration of culture was almost $1.7 \cdot 10^9$ CFU/mL) the same method was applied by using MRS agar instead of nutrient agar. However, the second layer of MRS agar was also poured onto these petri plates after placing the films. All petri plates were incubated for 3–4 days at 37 °C, and the formation of zones was checked and their diameter was measured.

Results and Discussion

Crude exopolysaccharides and films

In the literature, alcohol precipitated exopolysaccharides from *A. pullulans* fermentation have sometimes been referred to as pullulan without assaying their composition. However, depending on the morphological forms of *A. pullulans* during fermentation and the fermentation conditions, different forms of pullulan, β-glucans, acidic polysaccharides and heteropolysaccharides may be present in the recovered exopolysaccharides (*16, 17*). In this study, the blend of alcohol precipitated exopolysaccharides obtained from three different fermentations contained 59 % of pullulan. Previously, by using the same *A. pullulans* strain and fermentation conditions,

we had obtained 85 % of pullulan in ethanol precipitated crude exopolysaccharides (*18*). It is possible that this difference was due to the slight changes in the morphological form of *A. pullulans*. Champbell *et al*. (*16*) reported marked modifications in the pullulan contents of fermentation media in short periods of time, between 72nd and 84th hour of fermentation. These workers thought that the marked drop in pullulan content was related to the increased activity of the exopolysaccharide degrading enzymes of *A. pullulans* during fermentation. Thus, it is also likely that the variations in the pullulan content of different fermentations were due to modifications in the enzyme synthesis mechanisms of *A. pullulans*. On the other hand, because *A. pullulans* P56 is a melanin-deficient strain, the films obtained were colourless and transparent (Fig. 1). The films were also water-soluble and they did not brake up when they were folded. The incorporation of lysozyme and disodium EDTA·$2H_2O$ into the films did not cause a detectable change in colour, transparency and solubility of the films. High concentration of sucrose (at 10 mg/cm^2) incorporation, on the other hand, made the films very sticky and prevented their peeling from the plastic petri plates. Further studies are needed to determine the exact changes in the mechanical properties of films by incorporation of different ingredients.

Partially purified lysozyme

In the studies related to antimicrobial films, most of the workers used commercial lysozyme obtained by the classical repeated salt crystallization method, which requires a week until the enzyme is obtained with sufficient purity. Commonly used commercial lysozymes are pure, reported to contain only 1–6 % of protein impurities (*22*), and they have a very high enzyme activity (between 20 000–100 000 U/mg). However, for the application of lysozyme in food industry the use of partially purified lysozyme preparations obtained by some faster methods may be more economical. For this reason, in this study we used partially purified lysozyme obtained simply by ethanol precipitation of nonlysozyme proteins such as ovalbumin and conalbumin in the egg white.

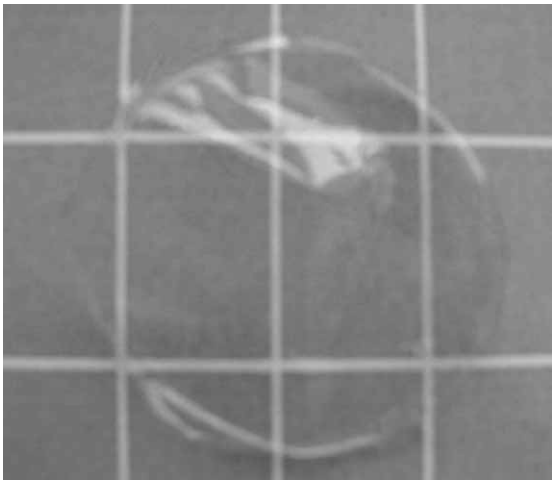

Fig. 1. A film obtained from crude exopolysaccharides of *A. pullulans* (the white lines at the background help seeing the transparency)

As seen in Table 1, by using the standard assay conditions applied in this study, the recovery and specific activity of partially purified lysozyme were determined as 114 % and 4095 U/mg, respectively. However, changes in assay conditions affected the partial purification parameters. For example, monitoring the purification process by using a reaction mixture of 2.3 mL of *Micrococcus lysodeikticus* cell suspension in buffer and 0.2 mL of enzyme extract determined the recovery and specific activity of the lysozyme as 64 % and 3526 U/mg, respectively. This was because of almost 50 and 15 % of higher and lower enzyme activities of the crude and partially purified enzymes under the altered conditions, respectively. Thus, although the protective effect of ethanol on lysozyme was reported by Jiang *et al*. (*20*), some kinetic changes may occur in the enzyme, depending on conformational changes caused by the ethanol treatment.

Effect of lysozyme concentration and film ingredients on lysozyme activity remained in films

As seen in Fig. 2, the presence of disodium EDTA ·$2H_2O$ alone at 52 μg/cm^2 or disodium EDTA·$2H_2O$ at 520 μg/cm^2 in combination with sucrose at 10 mg/cm^2 did not affect significantly the lysozyme activity determined for the films. When lysozyme was incorporated into films at 100 μg/cm^2, the recovered activity after film making varied between 23 and 55 %. Increase of the incorporated lysozyme to 260 μg/cm^2, on the other hand, increased the recovery of activity after the film making to 65–70 %. Above 260 μg/cm^2 of lysozyme incorporation, the activity of the enzyme measured in the films either increased slightly or remained almost constant. Thus, at high lysozyme concentrations, this reduced the recovery of activity down to almost 25 %. The results of this study clearly showed that the incorporation of different ingredients did not cause significant differences in the initial lysozyme activity of the films. Thus, it is likely that the reduction in lysozyme activity

Table 1. Summary of the partial purification of lysozyme from hen egg white

Steps	*V*	Total activity	Total protein	Specific activity	Recovery	Purity
	mL	U	mg	U/mg	%	(fold)
Dilution of egg white with 0.05 M NaCl						
	150	2 568 750	8162	315	100	1
pH adjustment / treatment with ethanol / incubation / dialysis						
	394	2 919 540	713	4095	114	13

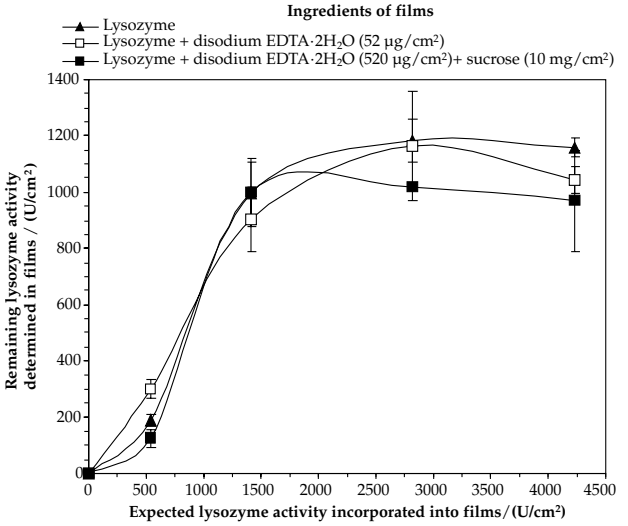

Fig. 2. Effect of lysozyme concentration and different ingredients on lysozyme activity remained in films

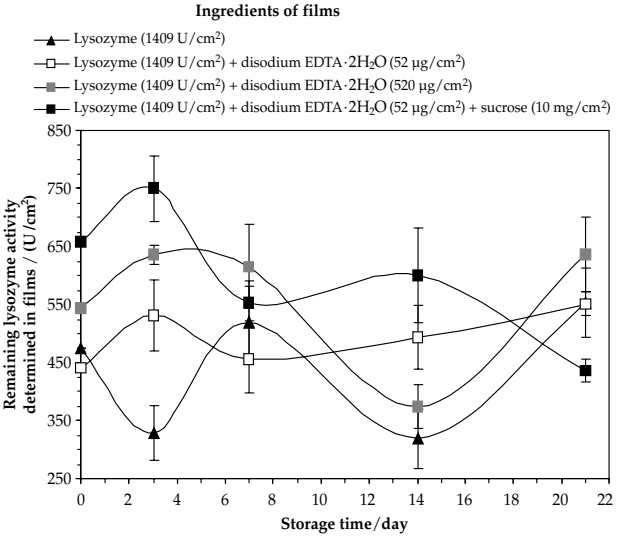

Fig. 3. Effect of cold storage and different ingredients on lysozyme activity remained in films

by film making was related with the interactions between the enzyme and the crude exopolysaccharides or the other impurities coming from the fermentation medium.

Effect of cold storage on lysozyme activity remained in films

During 3 weeks of cold storage at 4 °C, significant fluctuations were observed in the lysozyme activity of the films (Fig. 3). Such fluctuations in the activity of enzymes are observed very frequently when they are stored in crude media. These changes may be due to reversible modifications in enzyme conformation, association-dissociation reactions formed between the enzyme and protein or carbohydrate molecules or formation of some enzyme activating or inhibitory products during storage. In films, except those containing only lysozyme, a slight increase was observed in the enzyme activity after 3 days of cold storage. In films containing only lysozyme, the increase in the enzyme activity occurred in the first week of the cold storage, following a sharp drop in the enzyme activity during the first 3 days of cold storage. Following the limited activation in all films, the enzyme showed moderate to significant drops in activity. However, because of the repeated activation of the enzyme in sucrose-free films, the final activity of the enzyme in films was slightly over the initial activity of the enzyme at the end of 3 weeks. In films containing sucrose, on the other hand, the enzyme did not show a final activation and the continued drop resulted with the loss of almost 35 % of the initial enzyme activity in the films. In literature, sucrose was reported to have a stabilizing effect on the conformation of different proteins including lysozyme. The stabilizing effect of this sugar is observed both in aqueous solutions of proteins (*23*) and in their lyophilized or freeze dried forms (*24*). Liao *et al.* (*24*) attributed the stabilizing effect of sucrose in dried conditions to its ability to make hydrogen bond with the lysozyme. It is hard to estimate the complex reactions causing a partial loss of enzyme activity in sucrose containing films at the end of 21 days of storage. However, it is likely that sucrose had limited the formation of conformational changes in the enzyme that caused the repeated activation.

Antimicrobial activity of films

In antimicrobial tests for controls and other films containing ineffective concentrations of lysozyme or disodum EDTA·$2H_2O$, extensive microbial growth was observed at film locations and remaining agar surface (Table 2). As expected, the films containing only lysozyme did not show any antimicrobial activity on *E coli*. In 260 μg/cm² (expected activity: 1409 U/cm²) of lysozyme and 52 μg/cm² of disodium EDTA·$2H_2O$ incorporated films, no zones were observed in petri plates inoculated with *E. coli*. However, in 260 or 780 μg/cm² of lysozyme and 520 or 52 μg/cm² of disodium EDTA·$2H_2O$ incorporated films, respectively, unclear zones, observed in 2–3 of the 9 films tested, suggested a slight inhibition. In contrast, clear zones observed in all disc locations against *E. coli* for 780 μg/cm² (expected activity: 4227 U/cm²) of lysozyme and 520 μg/cm² of disodium EDTA·$2H_2O$ incorporated films indicated strong antimicrobial activity at these concentrations (Fig. 4). As indicated above, when more than 260 μg/cm² of lysozyme were incorporated into films, no significant differences were determined in the lysozyme activities of the films. Thus, it is clear that the lysozyme activity measured in films may not always be an indication of strong antimicrobial activity.

On the other hand, 260 or 780 μg/cm² of lysozyme incorporated films did not inhibit *L. plantarum*. For *L. plantarum*, the minimum inhibitory concentration of commercial lysozyme (Sigma Chem. Co.) incorporated to zein films was reported as 50–100 μg per 0.86-cm diameter discs (*5*). Considering the reported activity of Sigma lysozyme (50 000 U/mg), the activity of these discs (2500–5000 U) should be close to that of discs of our 780 μg/cm² of lysozyme incorporated films. However, it seems that partial inactivation of incorporated lysozyme in our films prevented inhibition at this concentration.

Table 2. Antimicrobial effects of films from crude exopolysaccharides of *A. pullulans* at different lysozyme and disodium EDTA · $2H_2O$ concentrations

Film no.	μg/cm^2		μg /film discs tested		Description of film disc locations*
	lysozyme	disodium EDTA · $2H_2O$	lysozyme	disodium EDTA · $2H_2O$	
					E. coli
1	–	–	–	–	No zones
2	260 (1409)[a]		166 (902)[b]		No zones
3	780 (4227)		499 (2705)		No zones
4	260 (1409)	52	166 (902)	33	No zones
5	260 (1409)	520	166 (902)	333	No clear zones / less microbial growth at 3 disc locations
6	780 (4227)	52	499 (2705)	33	No clear zones / less microbial growth at 2 disc locations
7	780 (4227)	520	499 (2705)	333	Clear zones at film locations (average area = 1.1 cm^2)
					L. plantarum
8	–	–	–	–	No zones
9	260 (1409)	–	166 (902)	–	No zones
10	780 (4227)	–	499 (2705)	–	No zones

[a]Parentheses in this column are expected lysozyme activities in films as U/cm^2
[b]Parentheses in this column are expected lysozyme activities in film discs as U
*9 discs were tested for each film

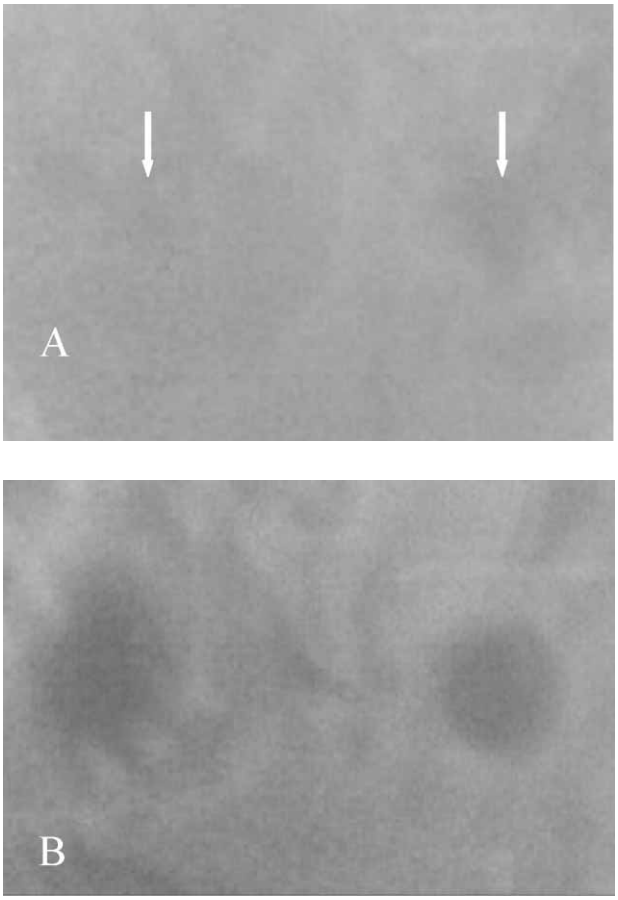

Fig. 4. Photographs of nutrient agar surfaces showing the antibacterial effect of lysozyme and disodium EDTA · $2H_2O$ incorporated films on *E. coli*. (**A.** discs contained 902 U of lysozyme and 333 μg of disodium EDTA · $2H_2O$; **B.** discs contained 2705 U of lysozyme and 333 μg of disodium EDTA · $2H_2O$

Conclusions

Partially purified lysozyme showed sufficient stability in the prepared films for 21 days with or without the presence of disodium EDTA · $2H_2O$ during cold storage. The lysozyme incorporated films did not show any inhibitory effect on *L. plantarum* at the studied concentrations. However, when combined with disodium EDTA · $2H_2O$ they exhibited antimicrobial activity against *E. coli*. Due to the crude nature of exopolysaccharides, the antimicrobial films obtained cannot directly be used as edible films without toxicological testing. However, they can be used as biodegradable coatings for fruits and vegetables with inedible skin to reduce the recently increased foodborne illnesses in these products contaminated by *E. coli* and other pathogens from soil and manure composts (*25–27*). Further studies are now continued in our laboratory to determine the effects of this antimicrobial system on selected fruits and vegetables.

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Proizvodnja antimikrobnih filmova ugradnjom djelomično pročišćenog lizozima u biorazgradive filmove sirovih egzopolisaharida dobivenih fermentacijom od *Aureobasidium pullulans*

Sažetak

Antimikrobni filmovi dobiveni su ugradnjom djelomično pročišćenog lizozima u filmove sirovih egzopolisaharida (59 % pululana) proizvedenih fermentacijom *Aureobasidium pullulans*. Dobiveni filmovi sadržavali su 100, 260, 520 i 780 μg/cm^2 lizozima, a imali su od 23 do 70 % očekivane enzimske aktivnosti. Filmovi pripravljeni ugradnjom 260 μg/cm^2 lizozima (1409 U/ cm^2) imali su najveću preostalu enzimsku aktivnost (65–70 %). Dodatak dinatrijeva EDTA·2H_2O i saharoze nije bitno utjecao na početnu lizozimsku aktivnost filmova. Uz dodatak 52 ili 520 μg/cm^2 dinatrijeva EDTA·2H_2O ili bez njega lizozimska aktivnost je bila dovoljno stabilna u filmovima čuvanim na hladnom tijekom 21 dana. Prisutnost 10 mg/cm^2 saharoze u filmovima destabilizirala je enzimsku aktivnost (skoro do 35 %) na kraju skladištenja. Zajedničkom ugradnjom 780 μg/cm^2 lizozima (4227 U/cm^2) i 520 μg/cm^2 dinatrijeva EDTA·2H_2O dobiven je antimikrobni film djelotvoran na *Escherichia coli.* Međutim, filmovi s prije navedenim koncentracijama lizozima nisu pokazivali antimikrobnu aktivnost prema *Lactobacillus plantarum.* Dobiveni rezultati jasno pokazuju da se djelomično pročišćeni lizozim i sirovi egzopolisaharidi od *Aureobasidium pullulans* mogu koristiti za pripravu antimikrobnih filmova kako bi se povećala sigurnost hrane.