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Antimicrobial Activity of the Cell Organelles, Lysosomes, Isolated from Egg White

Yoon, Jihee[1], Jae-Min Park[2], Ki-Ju Kim[1], Yang-Hoon Kim[2]*, and Jiho Min[1]*

[1]*Graduate School of Semiconductor and Chemical Engineering, Chonbuk National University, Jeonju 561-756, Korea*
[2]*School of Life Science, Chungbuk National University, Cheongju 361-763, Korea*

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Lysosomes, as a cell organelle type, are safe biological control agents that may be possible replacements for chemical antimicrobial agents because they are simply isolated from egg white. In this study, it was found that the lysosomes isolated from egg white exhibited pH-dependent antimicrobial activity, with the optimal activity found at pH 6.0. The efficiency of lysosomes in inhibiting bacterial growth and activity was evaluated over a 12-h treatment period. Seven different microorganisms were used as bacterial strains, and the lysosomes showed a significant antimicrobial effect against all strains. In addition, the antimicrobial activity was maintained for 100 days, and there did not appear to be any resistance of *E. coli* to the lysosomal activity up to the eighth culture. However, the lysosomes did not affect the viability of mammalian cells, suggesting the biocompatibility of lysosomes. These highly effective lysosomes have a bright future in the application of novel antimicrobial sources as a cell organelle type.

Keywords: Lysosomes, cell organelle, antimicrobial activity, egg white

Lysozymes are used in a variety of food and pharmaceutical products on account of its antimicrobial properties [6, 10]. Lysosomes are cell organelles containing 50–60 hydrolases that represent the cellular site for bulk macromolecule degradation [2, 15]. The activities of lysosomes mediate several processes in cell feeding and antimicrobial defense, which involve lysosome fusion with endocytosis and autophagy [2, 8, 15]. Lysosomes and their enzymes are capable of cellular digestion, and tissue destruction and remodeling [1]. The autophagic and heterophagic functions of lysosomes are responsible for the digestion and removal of excess intracellular and extracellular materials [2, 8].

In addition, lysosomes can release their contents, which contain a variety of hydrolytic enzymes that are capable of digesting the intra and extracellular matrices [2, 5]. Bacteria cell walls are degraded by the lysozymes released from lysosomes, which function as hydrolytic enzymes [4, 5]. If the lysosomes come in contact with the bacteria with a cell wall undergoing *N*-acetylation, the bacteria might be hydrolyzed as a result of the antimicrobial activity of the lysosomal enzyme released from the lysosomes [4, 5]. In contrast, lysosomes cannot degrade the bacteria cell walls if the functional group of the cell wall is converted from *N*-acetylation to *O*-acylation [4, 5].

This study examined the antimicrobial activity of lysosomes as a cell organelle. Recently, lysosomes as a cell organelle isolated from *Saccharomyces cerevisiae* were used to analyze antimicrobial activity against several microorganisms [15]. The lysosomes' antimicrobial activity is significanly enhanced using oxidative stress such as exposure of hydrogen peroxide in *S. cerevisiae* [15].

In this study, a solvent for the lysosomes that maintains their antimicrobial activity was selected, and here, we used lysosomes isolated from white egg. In addition, several reaction conditions to optimize the lysosomal activity were examined, such as the concentration of lysosomes to kill several microorganisms, the pH of the dissolving solvent, and the reaction temperature. This paper reports for the first time that the lysosomes as a cell organelle isolated from egg white are potent antimicrobial agents against a variety of microorganisms.

Materials and Methods

Tested Microorganisms

The microorganisms used to evaluate the lysosomal activity were *Escherichia coli* Top10, *Xanthomonas oryzae* KACC10859, *Shigella*

**Corresponding author*
Phone: +82-43-261-3575; Fax: +82-43-264-9600;
E-mail: kyh@chungbuk.ac.kr
Phone: +82-63-270-2436; Fax: +82-63-270-2306;
E-mail: jihomin@chonbuk.ac.kr

flexneri, *Streptomyces albus* KCTC1082, *Deinococcus radiophilus* ATCC27603, and *Corynebacterium glutamicum*, and *Saccharomyces cerevisiae* w303a as a fungus. Bovine aortic endothelial cells (BAECs) were isolated as the primary cell type and were cultured as previously described [11] and the BAECs were used to evaluate the biocompatibility of the lysosomes.

Lysosomes Isolation from Egg White

The lyososomes were isolated from egg white using cell fraction methods [7]. The yolk was separated from the white component of the eggs and discarded. The white component of hen eggs was homogenized until the viscosity of the egg white decreased significantly. Two particulate fractions were obtained by centrifuging the homogenate successively for 5 min at 500 ×*g* (unbroken cells and nuclei), and 30 min at 20,000 ×*g* (lysosomal fraction). The remaining pellet was used to determine the antimicrobial activity of the fraction [13].

Antimicrobial Test

An antimicrobial test was performed to confirm the antimicrobial activity of the lysosomes. After the cell culture at OD_{600} reached 0.6–0.7, the cultures were diluted to 10^{-6} cells/ml in sterile water. One hundred μl of the cell cultures with the lysosomes in a 100 mM sodium phosphate buffer (900 μl) was spread over an LB plate medium. All the experiments were carried out in an incubator at 37°C for 12 h. The antimicrobial activity of the lysosomes and its derivatives was measured using the colony counts [4, 13]. In order to evaluate the adaptation of *E. coli* to the antimicrobial activity of lysosomes, the cell culture was diluted to 10^{-6} with sterile water when the *E. coli* culture reached an OD_{600} of 0.7. The diluted *E. coli* and 20% lysosomes were mixed at a 1:9 ratio. One hundred μl of the mixture was spread over an agar plate and incubated for 12 h at 37°C. If colony growth was observed on the agar plate, a single colony was diluted to 10^{-6} with sterile water. This procedure was repeated a total of eight times.

Scanning Electron Microscopy (SEM) Sample Preparation

The sample was first fixed with 2.5% gluteraldehyde/0.1 M phosphate buffer for 4 h at 4°C. A second fixation procedure in 1% osmium tetraoxide/0.1 M phosphate buffer was then carried out overnight at 4°C. The fixed samples were rinsed with phosphate buffer for 10–15 min. After buffering, the sample was submerged in a graded series of ethanol (30%, 50%, 70%, 80%, 90%) for 20 min each, and then submerged in 100% ethanol three times at 20-min intervals to ensure full dehydration. Since CO_2 is miscible in ethanol, the samples were critical-point dried for approximately 40 min. Critical-point drying removes ethanol without any surface tension forces, which can distort the sample. The sample was then mounted onto a metal stub with double-sided carbon tape. Finally, a thin layer of metal (gold and palladium) was deposited onto the sample using an automated sputter coater.

Labeling of the Lysosomes with Lyso Tracker

The cells were grown in a cell culture dish, rinsed with 1× PBS, and stained with 1 nM Lyso Tracker Green DND-26 (Molecular Probes, Leiden, The Netherlands) in the medium without serum for 30 min at room temperature. Subsequently, the cells were washed with PBS, fixed with 70% ethanol in PBS for 2 min at room temperature, and flattened on an agar overlay [9].

Data Analysis

All the data were obtained from three independent samples carried out simultaneously for error analysis, and the results are shown along with the standard deviation and the correlation between the cell mortality under several experimental conditions. The data were analyzed using Sigma Plot (SPS Chicago, IL. U.S.A.). A p value <0.05 was considered significant.

RESULTS AND DISCUSSION

Optimization of Antimicrobial Conditions of Lysosomes Isolated from Egg White

Three important factors were considered when evaluating the antimicrobial activity of lysosomes isolated from egg white: the concentration of lysosomes, pH conditions of the solvent to dissolve the lysosomes, and the reaction temperature of the lysosomes needed to kill the bacteria. Fig. 1 shows the mortality of *E. coli* measured at 12 h after being treated with the lysosomes. Fig. 1A shows that the cell mortality increased in a concentration-dependent manner up to 20% of lysosomes. However, further increases

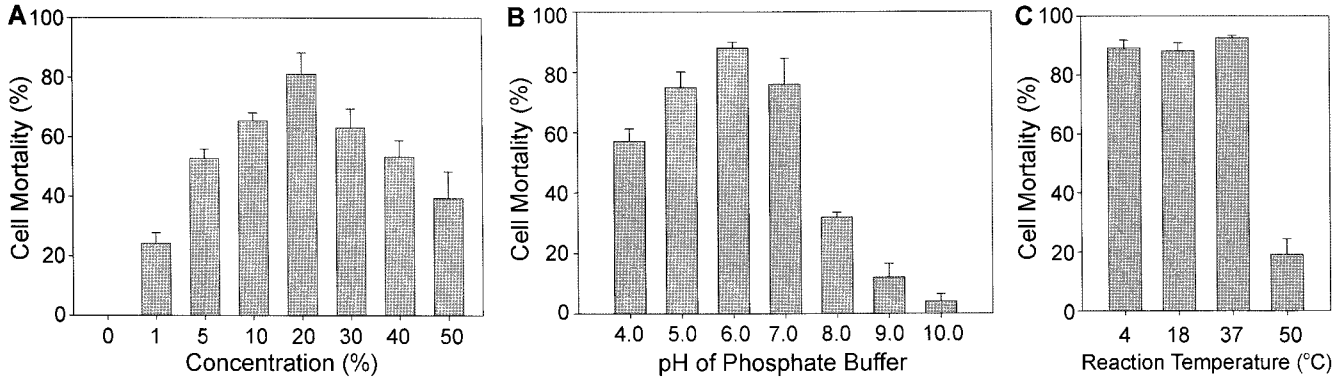

Fig. 1. Experimental conditions used to evaluate the antimicrobial activity of the lysosomes isolated from egg white.
E. coli cells were treated with 8 different concentrations of lysosomes in phosphate buffer (pH 6.0) (**A**). The mortality of *E. coli* was examined using 20% lysosomes dissolved in the phosphate buffer at different pHs (**B**), and reacted at 4 different reaction temperatures (**C**).

in the lysosome concentration resulted in a decrease in antimicrobial activity. There might be some limitations in the antimicrobial activity of lysosomes during their dissolution in the phosphate buffer owing to the high viscosity of the lysosomes.

Various enzymes inside the lysosomes may be damaged under alkaline conditions [1, 14] because the interior pH of the lysosomes is 4.8 [3, 12]. Therefore, this study examined the effect of pH on the antimicrobial activity of lysosomes. The antimicrobial activity was measured after dissolving the lysosomes to a concentration of 20% in phosphate buffer at different pHs. The relationship between the pH and antimicrobial activity of lysosomes was determined and the results are shown in Fig. 1B. The cell mortality from the lysosomes at pH 4.0, 5.0, and 6.0 was 57.2%, 75.0%, and 88.0%, respectively. The antimicrobial activity of the lysosomes in acidic conditions increased with increasing pH. However, the antimicrobial activity was inversely proportional to the increase in pH under alkaline conditions. Therefore, the lysosomes in phosphate buffer at pH 6.0 showed optimal antimicrobial activity.

In addition, 4 different reaction temperatures were used to examine the activity of lysosomes during the antimicrobial reaction. As shown in Fig. 1C, the antimicrobial activity of lysosomes was unaffected by the reaction temperature (4°C, 18°C, and 37°C). However, the antimicrobial activity deteriorated at 50°C owing to the decreasing activity of the lysosomal enzymes at high temperatures.

Evaluation of Lysosomes as a Novel Antimicrobial Agent

The effect of long-term storage on the activity of lysosomes was also examined to evaluate the possibility of lysosomes as a commercialized product. In this study, the lysosomes were stored at different storage temperatures (4°C or 20°C) to determine the effects of temperature on the antimicrobial activity during long-term storage. As shown in Fig. 2A, the antimicrobial activity was affected significantly by the storage time after 100 days. The cell mortality of *E. coli* treated with the lysosomes was maintained for up to 65.4% of the initial cell mortality, but the extent of cell mortality was decreased dramatically to 39.1% with increasing storage time. This suggests that the antimicrobial activity of lysosomes can be maintained for up to 100 days, after being isolated from egg white.

The antimicrobial effect of the lysosomes isolated from the egg white was observed in different strains including Gram-positive, Gram-negative, and eukaryote cells. As shown in Fig. 2B, the growths of *D. radiophilus*, *C. glutamicum*, *X. oryzae*, *S. flexneri*, and *S. cerevisiae*, including *E. coli*, were influenced by the lysosome treatment, except for *S. albus*. In the case of *S. albus*, the antimicrobial effect was lower than the other species. This suggests that the lysosomal activity against some strains forming bacterial spores is relatively low.

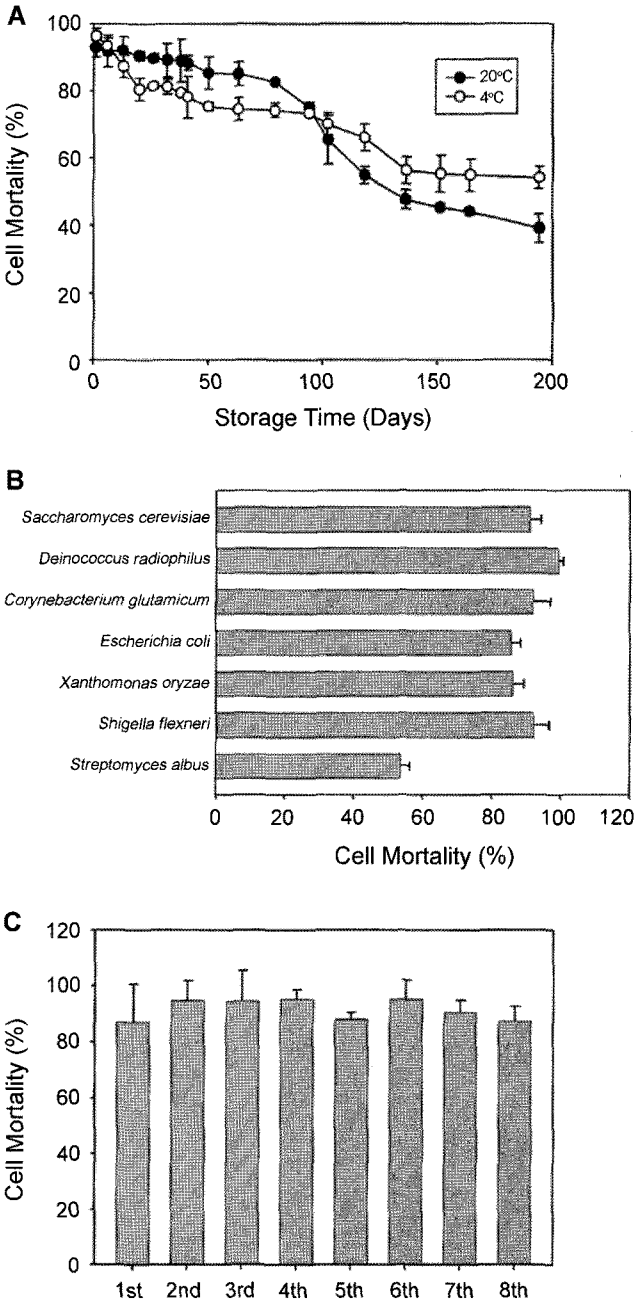

Fig. 2. Evaluation of the antimicrobial activity of lysosomes from eggs white.

A. Effects of the storage time and temperature on the cell mortality of *E. coli*. **B.** Mortality of seven strains treated with the lysosomes. **C.** Antimicrobial resistance of *E. coli* to the lysosomes after repetitive use, a total of eight times. The used concentration of lysosomes in phosphate buffer (pH 6.0) was 20%.

Microorganisms adapt more quickly to antimicrobial agents than it takes to discover new ones. Therefore, as mentioned in the Materials and Methods section, experiments were carried out to determine the adaptive and resistant responses that occur after *E. coli* cells were treated by the lysosomes from egg white. The lysosome-treated cells

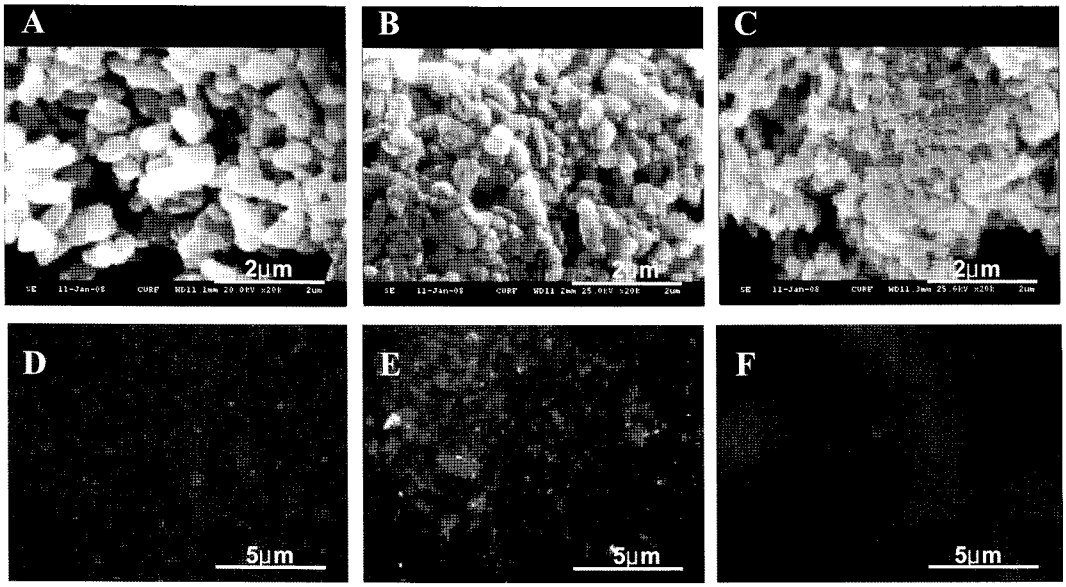

Fig. 3. Images of (**A**, **B**, **C**) scanning electron microscopy (SEM), and (**D**, **E**, **F**) fluorescent microscopy using Lyso Tracker dye. Solutions of (**A**, **D**) pH 4.0, (**B**, **E**) pH 6.0, (**C**, **F**) pH 10.0 in phosphate buffer were used.

from the first culture were transferred to a second culture for a second lysosomes treatment. The experiments were conducted eight times, as shown in Fig. 2C. There did not appear to be any adaptation or resistance of *E. coli* to the lysosomal activity up to the eighth culture. Therefore, the antimicrobial activity of lysosomes was maintained from the first treatment to the eighth treatment.

Effect of Solvent pH on the Antimicrobial Activity

Lysosomes isolated from the egg white were prepared in 100 mM sodium phosphate buffers at pH 4.0, 6.0, and 10.0 as a dissolving solvent. In the previous figure, the lysosomes at pH 6.0 had greater antimicrobial activity than under the other pH conditions. Therefore, the effect of pH on the lysosomal activity was analyzed by observing the morphology of lysosomes by SEM and the fluorescent intensity of the lysosomes stained with a Lyso tracker dye. At higher pH, the lysosomes decreased in size, suggesting inactivation of the lysosomal enzymes under alkaline conditions (Fig. 3A, 3B, and 3C). On the other hand, many lysosomal enzymes were activated and transported into the lysosomes at pH 6.0, but not at pH 4.0, as determined from the fluorescent intensities of the lysosomes under different pH conditions (Fig. 3D, 3E, and 3F).

Biocompatibility of Lysosomes as an Antimicrobial Agent

Antimicrobial agents are used widely in agriculture and industry, and humans are always exposed to their side effects. This study evaluated the biocompatibility of lysosomes based on the cell viability of BAECs, with chemical antimicrobial agents, as a commercialized product, being tested for comparison. In the case of 20% lysosomes, which had the highest antimicrobial activity (Fig. 4), the lysosomes did not affect the mammalian cells, suggesting the biocompatibility of lysosomes. However, the chemical antimicrobial agents caused cell death. Therefore, the lysosomes are more biocompatible than the other antimicrobial agents.

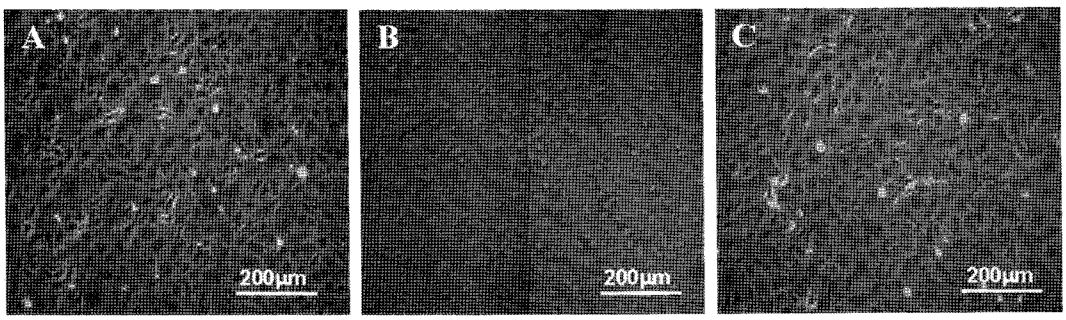

Fig. 4. Evaluation of the lysosomes as a biocompatible agent.
A. Control; **B**. Commercialized chemical agent A; and **C**. 20% lysosome.

Lysosomes isolated from egg white were used to efficiently evaluate the antimicrobial activity at different pHs of the dissolving solvent and reaction conditions. This study provides the first evidence of the antimicrobial activity of lysosomes isolated from egg white, not lysozymes, and in addition, the antimicrobial activity of lysosomes was evaluated in this study using simple conditions, long duration, no resistance responses, and nontoxic antimicrobial agents. These results suggest that lysosomes can be a new antimicrobial agent, as they are not toxic. Therefore, we expect high effectiveness if they are used as an antibiotic.

Acknowledgments

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